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METAXENIA IN COTTON[1]

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INTRODUCTION

It has been observed at the United States Field Station, Sacaton, Ariz., that well-grown plants of inbred families of Egyptian cotton show occasional variations of considerable magnitude in the length of the lint borne on individual seeds. In the same boll one or more seeds may be found on which the lint is as much as one-eighth inch shorter than the average. The discovery by Swingle (*9*)[2] and Nixon (*7*, *8*) of "metaxenia," or immediate effect of pollen on tissues of the mother plant, in the date palm suggested the possibility that the variations observed in cotton may in some cases result from cross-pollination. Search of the literature having failed to reveal any mention of what might be considered metaxenia effects in the cotton plant, it was decided to test the matter experimentally.

EXPERIMENT IN 1928

MATERIAL AND METHODS

Two very distinct types of cotton, Egyptian (*Gossypium barbadense* L. ?) and Hopi (*G. hopi* Lewton), which differ greatly in the length of time required for maturation of the boll, in length of lint, and in fuzziness of seeds, were selected for the experiment. The Egyptian type was represented by a family of the Pima variety which had been strictly inbred four generations and was very uniform in all of its characters. The Hopi type, a peculiar cotton formerly grown by certain Indian tribes in Arizona, was represented by the variety designated "Sacaton," a form once cultivated by the Pima Indians along the Gila River, which differs slightly from the typical form of the species grown by the Hopi Indians in northeastern Arizona. In this case also a uniform family, inbred at least four generations, was available for the experiment.

Flowers on four of the Pima plants were marked by tags showing the date of anthesis and were left to natural pollination by their own pollen and that of surrounding plants of the Egyptian type,[3] while on three other Pima plants the flowers were emasculated to prevent

[1] Received for publication Nov. 25, 1930; issued May, 1931.

[2] Reference is made by number (italic) to Literature Cited, p. 543.

[3] Previous investigation had shown that even when the conditions were optimum for determining the extent of cross-fertilization, i. e., when individual plants were grown isolated in a plot of another kind of cotton, only 12 per cent of the ovules on the Egyptian plants proved to have been cross-fertilized (*2*, *p. 9*, *Table 5*). In the experiment here described, the Pima plants were in the center of a border containing only plants of the Egyptian type, so that such cross-pollination as may have occurred could not have affected the results appreciably.

self-fertilization and were pollinated with pollen from plants of the Hopi family. These flowers also were marked with tags showing the date of anthesis. The plants that received Egyptian pollen were less favorably situated and somewhat smaller than those that received Hopi pollen.

Tagging of the flowers was begun on July 24 and ended September 5. Only the bolls that opened before the first frost were harvested, since frosted bolls open prematurely and hence are unsuitable for determination of the maturation period.

Table 1 gives the statistical constants for the characters determined on the material from the pollinations Pima × Pima and Pima × Hopi. These were: Boll period, lint length, lint index, seed fuzziness, number of seeds per boll, and weight of seeds. The boll period was recorded as the number of days from anthesis to opening of the boll. Lint length and seed fuzziness were indicated by grades, the latter having been determined after the samples were ginned. The lint index and the weight of the seeds were determined in grams, the latter after removal of the lint. In computing these values the weight of all the seeds and of all the lint in each boll was divided by the number of seeds therein, and the quotients were multiplied by 100 in order to avoid the use of small fractions of a gram.

TABLE 1.—*Statistical constants for characters determined on bolls from Pima cotton flowers naturally pollinated (Pima × Pima) and artificially cross-fertilized with Hopi pollen (Pima × Hopi)*

Character and population	Determinations	Mean	Standard deviation	Coefficient of variation
Boll period:	*Number*	*Days*	*Days*	
Pima × Pima	248	62. 2±0. 33	7. 80	12. 5±0. 39
Pima × Hopi	146	61. 1± . 41	7. 38	12. 1± . 48
Difference		1. 1± . 53		. 4± . 62
Lint length:		*Grade*	*Grade*	
Pima × Pima	102	8. 0±0. 02	0. 35	4. 4±0. 21
Pima × Hopi	93	7. 4± . 03	. 40	5. 4± . 27
Difference		. 6± . 036		1. 0± . 34
Lint index:		*Grams*	*Grams*	
Pima × Pima	65	5. 31±0. 037	0. 44	8. 3±0. 49
Pima × Hopi	57	5. 38± . 038	. 42	7. 8± . 49
Difference		. 07± . 053		. 5± . 69
Seed fuzziness:		*Grade*	*Grade*	
Pima × Pima	102	6. 6±0. 08	1. 20	18. 2±0. 89
Pima × Hopi	93	5. 8± . 10	1. 37	23. 6± 1. 23
Difference		. 8± . 13		5. 4± 1. 52
Seeds per boll:		*Number*	*Number*	
Pima × Pima	102	16. 6±0. 21	3. 09	18. 6±0. 91
Pima × Hopi	93	16. 5± . 26	3. 64	22. 0± 1. 14
Difference		. 1± . 33		3. 4± 1. 46
Weight 100 seeds:		*Grams*	*Grams*	
Pima × Pima	101	12. 7±0. 06	0. 89	7. 0±0. 33
Pima × Hopi	93	13. 2± . 06	. 84	6. 4± . 31
Difference		. 5± . 085		. 6± . 45

It had been discovered in other populations of cotton plants that some of these characters are correlated, and this fact should be taken

into account in considering possible metaxenia effects. The correlations were therefore computed for all pairs of the characters determined in the two populations, with the exception of boll period, which was not recorded in such a way as to make it possible to identify the individual bolls on which the other characters were determined. The coefficients of correlation are given in Table 2.

TABLE 2.—*Correlations of characters determined in populations from the fertilizations Pima×Pima and Pima×Hopi in 1928* [a]

Character and population	Correlation with—				
	Lint length	Lint index	Seed fuzziness	Number of seeds	Weight of seeds
	r	r	r	r	r
Lint length:					
Pima×Pima		−0.027±0.083	+0.034±0.067	−0.057±0.067	+0.089±0.067
Pima×Hopi		−.210±.085	+.287±.064	−.084±.069	+.190±.067
Lint index:					
Pima×Pima	−0.027±0.083		−.297±.076	+.056±.083	+.217±.080
Pima×Hopi	−.210±.085		−.123±.088	+.262±.083	−.028±.089
Seed fuzziness:					
Pima×Pima	+.034±.067	−.297±.076		−.220±.064	+.360±.058
Pima×Hopi	+.287±.064	−.123±.088		+.158±.068	+.074±.069
Number of seeds:					
Pima×Pima	−.057±.067	+.056±.083	−.220±.064		−.350±.059
Pima×Hopi	−.084±.069	+.262±.083	+.158±.068		−.193±.067
Weight of seeds:					
Pima×Pima	+.089±.067	+.217±.080	+.360±.058	−.350±.059	
Pima×Hopi	+.190±.067	−.028±.089	+.074±.069	−.193±.067	

[a] The number of determinations is 101 for the population Pima×Pima and 93 for the population Pima×Hopi, except in the case of lint index, for which the numbers are 65 and 57, respectively.

INFLUENCE OF POLLEN ON BOLL PERIOD

Maturity of the boll being a somewhat indefinite phenomenon, an arbitrary standard based on the degree of separation of the carpels was adopted and was recorded photographically. Figure 1 illustrates the standard of maturity adopted. In the fall each boll from a tagged flower was gathered as soon as it reached this stage, and the date was marked on the tag that bore the date of anthesis. The boll period, or number of days from anthesis to maturity, is the difference between the two dates. (Fig. 1.)

The parental types differ greatly in the length of the boll period. Determinations in 1928 on bolls from naturally pollinated flowers gave a mean of 62.2 ± 0.33 days for Pima (248 bolls) and a mean of 44.4 ± 0.37 days for Hopi (130 bolls). The mean difference, 18 days, is 36 times its probable error. The statistical constants of the period of development of bolls from Pima flowers pollinated, respectively, with Pima and with Hopi pollen are given in Table 1.

The average period for bolls from Pima flowers cross-fertilized with Hopi pollen (Pima×Hopi) was one day shorter than for bolls from the mostly self-fertilized flowers of the same variety (Pima×Pima), but the difference is not significant, being only twice its probable error. A significant difference might have been observed if the bolls of the two populations had been exposed to the same conditions. Since the Pima plants on which the flowers were fertilized with Hopi pollen were more luxuriant and larger leafed, the resulting bolls were more shaded than the bolls from naturally pollinated, hence mostly self-fertilized, Pima flowers. It is a matter of every-day observation that bolls exposed to full sunlight open sooner than shaded bolls, so

it may be assumed that in this experiment the full effect of Hopi pollen in shortening the maturation period of the Pima bolls was not realized because of environmental factors working in the opposite direction.

INFLUENCE OF POLLEN ON LINT LENGTH

The length of the lint was determined on one seed from each of 195 bolls borne on Pima plants, of which 102 bolls were from naturally pollinated flowers and 93 bolls were from flowers cross-fertilized with Hopi pollen. No determinations were made in the bolls from naturally pollinated flowers on the Hopi plants, but the lint of this inbred family is rather uniformly about 22 mm. (seven-eighths of an

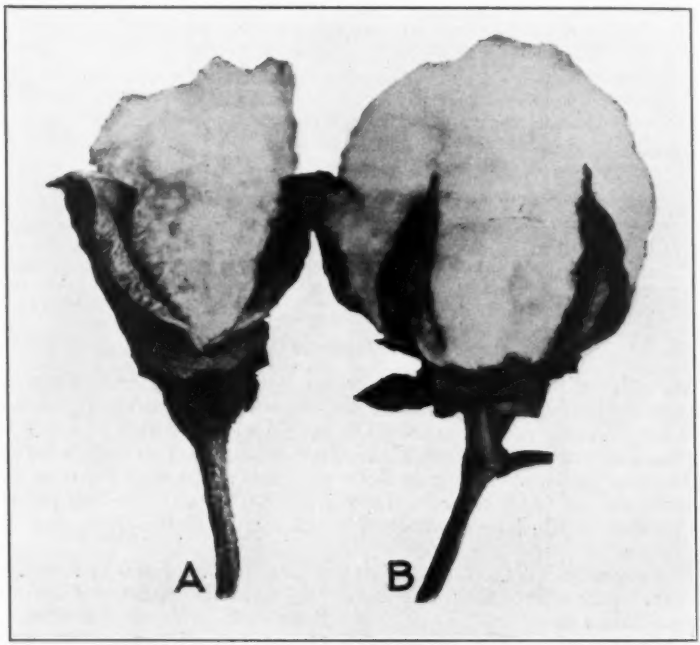

FIGURE 1.—Standard of maturity of bolls as used in this experiment: A, Pima Egyptian cotton; B, Durango upland cotton

inch) long, as compared with a mean length of 41 mm. (1⅝ inches) in Pima. (Fig. 2.) The pollination Pima × Pima was represented by bolls from three flowers of each of the 34 days on which flowers were tagged. In order to eliminate the influence of seasonal variations, the endeavor was made to have the 34 days equally represented also in the series of bolls from the pollination Pima × Hopi, but there were a few days toward the end of the period when fewer than three flowers were produced by the plants that received this pollination. Every day of the 34-day period is represented in both lots, however. There were only 9 more bolls in the lot representing the Pima × Pima pollination than in the lot representing the Pima × Hopi pollination, and the difference in number on a few days could not have affected

the result seriously. The lint length was graded by the method described by Kearney (*3, p. 10, fig. 2*). Since on this scale one grade corresponds to 3.2 mm. (one-eighth of an inch), the length in millimeters or inches represented by the mean of the determinations of grade in a population is easily computed.

Comparison of the means of lint length in Table 1 shows a reduction in length of the lint on the Pima plants resulting from fertilization with pollen of Hopi, a short-linted cotton, amounting to 0.6 grade, equivalent to 1.9 mm., or slightly more than one-sixteenth of an inch. The difference is highly significant, being 16 times its probable error.

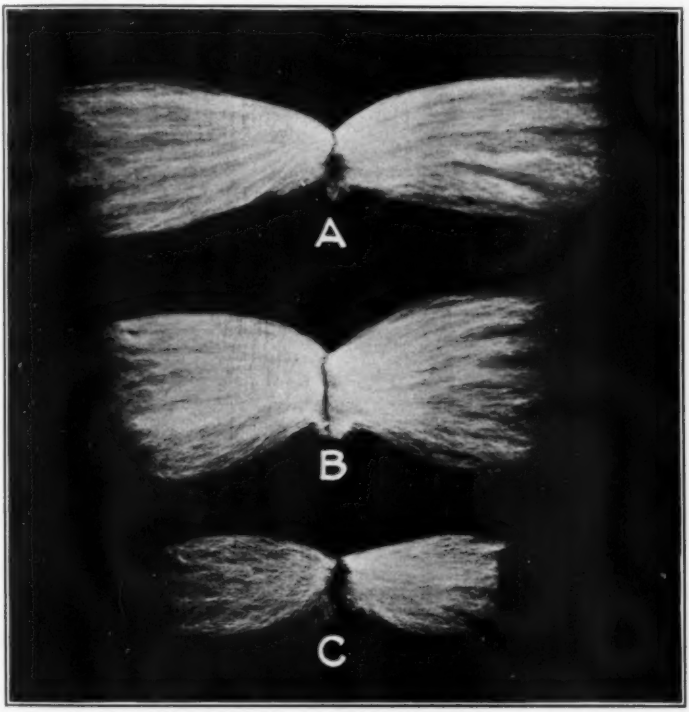

FIGURE 2.—Lint length of types of cotton used in this investigation: A, Pima Egyptian; B, Durango upland; C, Hopi. Natural size

The lint length in the bolls from the Hopi fertilization (Pima×Hopi) was less uniform than in the bolls from naturally fertilized flowers (Pima×Pima), but the difference between the coefficients of variation is barely 3 times its probable error.

Reference to Table 2 shows that lint length was significantly correlated with none of the other characters in the population Pima×Pima, but gave a significant positive correlation with seed fuzziness and possibly with seed weight in the population Pima×Hopi. Neither correlation would seem to affect the significance of the reduction in lint length by the Hopi pollen, which, as shown in Table 1, reduced

both the length of the lint and the amount of fuzz on the seeds while increasing the weight of the seeds. The absence of significant correlation between lint length and the other characters in the Pima × Pima population permits the inference that there is no general association of these characters which would necessitate regarding the effect of the kind of pollen on the length of the lint as an indirect one.

For the reason already mentioned, the full effect of the Hopi pollen in shortening the lint on the Pima plants probably was not realized in this experiment, the individual plants whose flowers received Hopi pollen having been grown under soil conditions conducive to greater length of the lint than were the individual plants whose flowers were fertilized by pollen of the same type (Egyptian).

INFLUENCE OF POLLEN ON LINT INDEX

Reference to Table 1 shows that the lint index was not significantly affected by the kind of pollen used, although the parental types, Pima and Hopi, differ considerably in abundance of the lint. (Fig. 2).

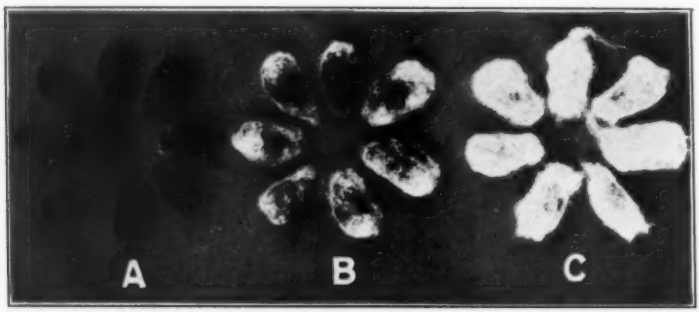

FIGURE 3.—Seeds after removal of lint: A, Hopi cotton; B, Pima cotton; C, Durango cotton. Natural size

INFLUENCE OF POLLEN SEED ON FUZZINESS

The family of Pima Egyptian cotton used in this experiment has decidedly fuzzy seeds, many of them having from one-half to nearly all of their surface covered with short hairs or fuzz, while the seeds of the Hopi family, after removal of the lint, are almost naked. (Fig. 3.) In view of this contrast, it was of interest to determine whether this character also is affected by the pollen. The degree of fuzziness was determined on the seeds from each of the bolls on which lint length was determined, the seeds having been compared with a series of nine samples representing the entire range of seed fuzziness observed in Egyptian cottons. The standard for grade 1 has the seeds naked except for a tuft of fuzz at the base and often a trace along the raphe, while in the standard for grade 9 most of the seeds are entirely or almost entirely covered with fuzz.

It is evident from the data in Table 1 that fertilization with pollen of Hopi, a smooth-seeded cotton, reduced the average quantity of fuzz on the seeds borne on the Pima plants by nearly a full grade. The difference is significant, being six times its probable error. As was also the case in lint length, fertilization with Hopi pollen increased the amount of variation in this character. The coefficient of variation

is significantly lower in the population Pima × Pima than in the population Pima × Hopi, the difference being 3.5 times its probable error. Reference to Table 2 shows that in the population Pima × Pima seed fuzziness was correlated significantly and negatively with lint index and with number of seeds, and significantly and positively with weight of seeds, while in the population Pima × Hopi the only significant correlation of this character was the positive one with lint length. It may be inferred from the fact that none of these pairs of characters showed significant correlation in both populations that the difference in seed fuzziness between the two populations was a direct effect of the kind of pollen and is not to be interpreted through the association of this with other characters.

NUMBER AND WEIGHT OF SEEDS

The number of seeds in the mature boll is determined by the initial number of ovules and by the degree of fertilization attained. No metaxenia effect is to be sought here, but the number of seeds should be considered in its relation to characters in regard to which metaxenia is indicated. Reference to Table 1 shows that the bolls of the two populations did not differ significantly in their mean number of seeds, so this could not have been a factor in the observed differences in other characters between the two populations.

Weight of the individual seeds, here expressed for convenience as mean weight per 100 seeds, is obviously not a satisfactory character for the detection of metaxenia in cotton, since in this plant almost the entire volume of the mature seed is occupied by the embryo, which is the young daughter plant rather than a part of the mother plant. The data in Table 1 show that the mean seed weight was significantly higher in the population Pima × Hopi than in the population Pima × Pima, the difference being nearly six times its probable error. It is probable that a heterosis or hybrid-vigor effect is involved, previous investigations having shown that the seeds from cross-fertilization of upland and Egyptian cottons are heavier than the seeds from fertilization within either type, and that the resulting F_1 plants show pronounced hybrid vigor, even in the early seedling stages.[4]

Of the two characters, lint length and seed fuzziness, which are believed to have shown metaxenia in this experiment, it is seen in Table 2 that the former was not significantly correlated with weight of seeds in the population Pima × Pima, while there was a positive but scarcely significant correlation in the population Pima × Hopi. Seed fuzziness was positively and significantly correlated with weight of seeds in Pima × Pima but not in Pima × Hopi. The absence of significant correlations of the same sign in both populations makes

[4] In an experiment performed at the Sacaton station in 1922, the results of which have not been published hitherto, flowers of Pima Egyptian cotton were emasculated and were pollinated, some with Pima pollen and some with pollen of the Lone Star variety of upland cotton. Reciprocal pollinations were made on emasculated flowers of Lone Star upland. Determinations of the weight of the individual seeds from the resulting bolls gave the following means:

	Gram
Pima × Pima	0.129 ± 0.0006
Lone Star × Lone Star	.126 ± .0006
Pima × Lone Star	.135 ± .0010
Lone Star × Pima	.135 ± .0040

The seeds from the reciprocal cross-fertilizations Pima × Lone Star and Lone Star × Pima had exactly the same mean weight, which was considerably greater than the weights of the seeds from the two fertilizations with like pollen.

it unlikely that the presumable metaxenia effect on lint length and seed fuzziness is associated with the difference in seed weight between the two populations, especially as the population which had the heavier seeds, Pima × Hopi, had the shorter lint and the smaller development of fuzz. (See Table 1.)

EXPERIMENT IN 1929

It was recognized that the experiment performed in 1928 was not as conclusive as could be desired, the flowers on the control plants having been left to natural pollination instead of having been strictly self-fertilized. There is good reason to believe that, since most of the ovules in naturally pollinated flowers are self-fertilized, and since the Pima plants in this experiment were surrounded by plants of this and other long-linted varieties of Egyptian cotton, such cross-pollination as may have occurred could not have vitiated the results appreciably. Nevertheless, before concluding that so remarkable a phenomenon as metaxenia occurs in cotton, it was desirable to obtain evidence from a more carefully controlled experiment.

MATERIAL AND METHODS

Three varieties, each belonging to a different species, were chosen for this experiment. These were the Pima variety of Egyptian cotton (*Gossypium barbadense* L. ?), the Durango variety of upland cotton (*G. hirsutum* L.), and Hopi cotton (*G. hopi* Lewton), the last being represented by the typical form grown by the Hopi Indians in northeastern Arizona. These varieties differ markedly in the time required for maturation of the boll and in length of the lint. The mean maturation period of Pima, as determined in 1928, is 62.2 days; of Durango as determined by King and his colleagues (*6*) in 1924, 47.4 days; and of Hopi, as determined in 1928, 44.4 days. The length of the lint averages about 41 mm. (1⅝ inches) in Pima, 32 mm. (1¼ inches) in Durango, and 22 mm. (seven-eighths of an inch) in Hopi. Inbred and uniform families of the Pima and Hopi varieties and plants of the Durango variety grown from the purest commercial seed obtainable were used for the experiment. All pollinations were made on Durango plants, this variety being approximately intermediate in lint length and having a boll-maturation period somewhat longer than that of Hopi and much shorter than that of Pima.

Durango plants to the number of 25, showing a thrifty and uniform growth, were selected. All flower buds due to open the following day on these plants were emasculated each evening and bagged to exclude insects, thus preventing unguarded cross-pollination. Flower buds which were to furnish pollen were bagged at the same time on the Pima, Durango, and Hopi plants, this precaution having been taken in order to prevent any admixture of foreign grains in the pollen used. The pollinations were made the following morning between the hours of 10 a. m. and noon, care being taken to apply enough pollen to insure the maximum attainable degree of fertilization.[5]

It was important to eliminate the influence of seasonal and positional variations, to which the characters of the cotton plant are peculiarly subject. It is well known, for example, that the time required for maturation of the boll is many days shorter for flowers produced early in the season than for the late summer flowers (*6, p. 948*). Position on the plant is also a factor influencing maturity

[5] The writer is indebted to H. J. Fulton and Warren Hastings for valuable assistance in these operations.

to an important extent. A boll situated so as to receive full sunlight will sometimes mature in 10 days' less time than a boll, originating on the same date, that has been shaded at all times. Lint and seed characters also are affected by position, Kearney and Harrison (*5*) having found that in Pima cotton the lint is shorter and the seeds are fuzzier in bolls borne near the base of the plant than in bolls borne higher. Here there is obviously a correlation between positional and seasonal effect, the "bottom" bolls being produced by very early flowers and the upper bolls by later flowers.

To avoid the influence of changes in conditions with the advance of the season, the ideal plan would have been to give each of the three pollinations to an equal number of flowers on each of the Durango plants on each day of the experiment. Since none of the plants produced as many as three flowers every day, this ideal was unattainable. The plan adopted was to keep an accurate record of the pollinations performed daily and to follow a definite rotation. An example will show how the plan operated. Assuming that Durango plant No. 1 produced two flowers on the first day of the experiment, one flower on the second day, and three flowers on the third day and that on the first day one flower was pollinated with Pima and one with Hopi pollen, then the one flower produced on the second day would be pollinated with Durango pollen, and one each of the three flowers produced on the third day would receive Pima, Hopi, and Durango pollen, respectively. It is believed that this procedure, which was followed systematically throughout the experiment, equalized the influence of both seasonal and positional variations and that the several lots of bolls representing the different pollinations were strictly comparable.

The first pollinations were made on July 19 and the last from which bolls matured before frost were made on September 5. The number of days on which pollinations were made during this period was 49. Owing to the lateness of the spring, it so happened that this period represented practically all of the active fruiting life of the Durango variety in 1929. Bolls that had not reached the accepted standard of maturity before frost occurred were disregarded.

All of the characters determined in the earlier experiment were measured in the experiment of 1929 with the exception of seed fuzziness. Owing to the fact that the female parent, Durango, has entirely fuzzy seeds (fig. 3), it was found impracticable to measure such variations as may have occurred in this character. The statistical constants of the five characters are given in Table 3, and the coefficients of the correlations among them in Table 4.

The populations on which the statistical constants were determined comprised bolls from 49 days of flowering in this experiment and from 44 days of flowering in the experiment of 1928. These periods represent a considerable range of seasonal conditions, and evidence has been obtained in other investigations that the length of the boll period, length of the lint, and degree of fuzziness of the seeds vary appreciably according to the time of flowering (*4*, *5*, *6*). These seasonal variations must have had the effect of increasing the standard deviations and probable errors. There is little doubt that the significance of the observed differences would have been greater if the bolls representing the several fertilizations had been produced during a shorter period of flowering.

TABLE 3.—*Statistical constants for characters determined on bolls from emasculated flowers of Durango upland cotton fertilized with pollen of Pima Egyptian, Durango upland, and Hopi cotton, respectively*

Character and population	Determinations	Mean	Standard deviation	Coefficient of variation
Boll period:	*Number*	*Days*	*Days*	
Durango×Pima	123	54.0±0.19	3.18	5.9±0.25
Durango×Durango	117	52.9± .20	3.16	6.0± .26
Durango×Hopi	124	52.5± .22	3.64	6.9± .30
Lint length:		*Grade*	*Grade*	
Durango×Pima	100	5.3± .05	.73	13.8± .67
Durango×Durango	100	4.9± .04	.64	13.2± .64
Durango×Hopi	100	4.6± .06	.90	19.4± .97
Lint index:		*Grams*	*Grams*	
Durango×Pima	100	6.49± .064	.95	14.6± .71
Durango×Durango	100	6.29± .051	.75	11.9± .58
Durango×Hopi	100	6.23± .062	.92	14.8± .72
Seeds per boll:		*Number*	*Number*	
Durango×Pima	100	24.4± .34	5.05	20.7±1.03
Durango×Durango	100	28.1± .30	4.51	16.3± .80
Durango×Hopi	100	28.1± .39	5.72	20.6±1.02
Weight 100 seeds:		*Grams*	*Grams*	
Durango×Pima	100	14.0± .11	1.62	11.6± .55
Durango×Durango	100	13.2± .08	1.18	9.0± .43
Durango×Hopi	100	13.0± .09	1.32	10.1± .48

TABLE 4.—*Correlations of characters determined in populations from the fertilizations Durango×Pima, Durango×Durango, and Durango×Hopi in 1929* [a]

Character and population	Correlation with—				
	Boll period	Lint length	Lint index	Number of seeds	Weight of seeds
Boll period:	*r*	*r*	*r*	*r*	*r*
Durango×Pima		+0.113±0.067	+0.258±0.063	+0.171±0.065	+0.047±0.067
Durango×Durango		−.060± .067	+.358± .059	+.073± .067	+.108± .067
Durango×Hopi		−.079± .067	+.135± .066	−.009± .067	+.006± .067
Lint length:					
Durango×Pima	+0.113±0.067		−.055± .067	−.011± .067	+.045± .067
Durango×Durango	−.060± .067		+.042± .067	−.128± .066	−.045± .067
Durango×Hopi	−.079± .067		+.012± .067	−.018± .067	[b]+.229± .067
Lint index:					
Durango×Pima	+.258± .063	−.055± .067		−.084± .067	+.231± .064
Durango×Durango	+.358± .059	+.042± .067		+.009± .067	+.259± .063
Durango×Hopi	+.135± .066	+.012± .067		−.126± .066	+.246± .063
Number of seeds:					
Durango×Pima	+.171± .065	−.011± .067	−.084± .067		−.457± .053
Durango×Durango	+.073± .067	−.128± .066	+.009± .067		−.273± .062
Durango×Hopi	−.009± .067	−.018± .067	−.126± .066		−.174± .065
Weight of seeds:					
Durango×Pima	+.047± .067	+.045± .067	+.231± .064	−.457± .053	
Durango×Durango	+.108± .067	−.045± .067	+.259± .063	−.273± .062	
Durango×Hopi	+.006± .067	[b]+.229± .064	+.246± .063	−.174± .065	

[a] The number of determinations is 100 for each character in each population.

[b] Omitting 5 very inferior bolls containing only 11 or 12 seeds and grading only 1.0 to 2.0 in lint length, or the remaining 95 bolls is reduced to + 0.072±0.069.

INFLUENCE OF POLLEN ON BOLL PERIOD

On the 25 Durango plants a total of 1,072 flowers were emasculated and pollinated, 359 with Pima pollen, 351 with Durango pollen, and 362 with Hopi pollen. The date of maturation of the boll was determined as in the experiment of 1928. The plants were inspected daily during the fall, and all bolls that had reached the stage of opening indicated by the standard of maturity were picked promptly.

The numbers of mature bolls obtained from the several pollinations were approximately equal, having been respectively 123, 117, and 124. The number maturing on the individual plants ranged from 7 to 25 and averaged 14.5. All bolls from a given pollination on all of the plants were taken as one array in computing the mean period of maturation.

The period from anthesis to opening of the boll on the Durango plants, as indicated by the data in Table 3, averaged 1.5 days longer from flowers fertilized with pollen of Pima Egyptian, a variety having a long maturation period, than from flowers fertilized with pollen of Hopi, a variety having a short maturation period. The difference is significant, being five times its probable error. Fertilization with Pima pollen also shows a significant retarding effect in comparison with the results of fertilization with pollen of Durango, which has a boll period somewhat longer than that of Hopi but much shorter than that of Pima. In this case the difference averages a little more than one day and is four times its probable error.

Fertilization with Hopi pollen showed a tendency to hasten the maturation of the bolls, but the mean difference between the populations Durango × Durango and Durango × Hopi is less than one-half day and is not significant, being little greater than its probable error. Nevertheless, it should be recalled that in the experiment of 1928 there was a slight although seemingly not significant shortening of the period of maturation of the bolls on Pima plants when the flowers were fertilized with Hopi pollen, as compared with the results of the fertilization Pima × Pima. The consistency of the results of the two experiments gives some warrant for the assumption that the pollen of the early-maturing Hopi variety tends to hasten the maturation of the boll when used in fertilizing the flowers of more slowly maturing types of cotton.

A tendency to greater variation in the boll period of individual bolls from fertilization with Hopi pollen than from the other fertilizations is indicated by comparison of the coefficients of variation, but the differences are not significant.

Only one of the other characters here considered, lint index, was significantly correlated with the boll period. As shown in Table 4, the coefficients were positive in all three populations and were significant in two of them. The relation between these characters does not decrease the probability that different pollens affect directly the length of the boll period, the correlation implying that the more slowly maturing bolls have more abundant lint on the seeds.

INFLUENCE OF POLLEN ON LINT LENGTH

The lint length was detemined, by the method used in the 1928 experiment, on each of 100 bolls resulting from each pollination. In selecting the bolls for these determinations it was sought to avoid variables due to individual differences among the plants and to development of the bolls at different times. The bolls of each lot, therefore, were selected so as to represent equally, as nearly as possible, the 25 plants for the entire period during which the pollinations were made. The relative length of lint of the three parent varieties is shown in Figure 2.

The data in Table 3 show that the lint on the Durango plants averaged 0.7 grade (2.2 mm.) longer in bolls from flowers fertilized

with pollen of Pima Egyptian, the longest linted of the three varieties, than in bolls from flowers fertilized with Hopi, the shortest-linted variety. The difference between the means is highly significant, being about nine times its probable error. An appreciable and significant lengthening of the lint on the Durango plants, due to fertilization with Pima pollen, is shown also by comparing the fertilizations Durango × Pima and Durango × Durango. The difference averages 0.4 grade (1.3 mm.) and is about six times its probable error. A smaller but significant shortening effect of the Hopi pollen is found when the fertilizations Durango × Durango and Durango × Hopi are compared. The difference between the means in this case is 0.3 grade (1.0 mm.) and is four times its probable error.

Since the weight of the seeds as well as the length of the lint was significantly greater in the population Durango × Pima than in the other populations, the possibility suggests itself that the greater length of the lint hairs was merely a response to better nutrition afforded by the heavier seeds. But in the experiment of 1928 the lint was significantly shorter, although the seeds were significantly heavier, in the population Pima × Hopi than in the population Pima × Pima. (Table 1.)

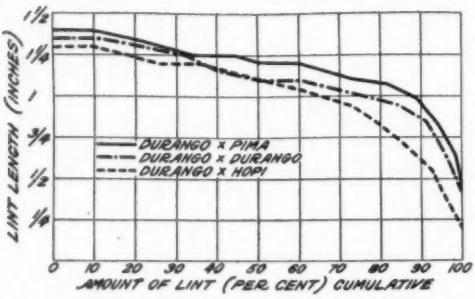

FIGURE 4.—Cumulative frequency curves showing the immediate effect of the several kinds of pollen on the length of lint on the Durango cotton plant

Comparison of the coefficients of variation in Table 3 shows no significant difference in the variation of lint length from boll to boll as between the fertilizations Durango × Pima and Durango × Durango, but the lint from the fertilization Durango × Hopi was significantly more variable than that from either of the other fertilizations.

The means of lint length as given in Table 3 are based on rather rough determinations of the length of the bulk of the fibers on individual seeds, one from each boll. The method gives a result essentially the same as in commercial classing for length. It is well known, however, that in the most uniform cottons, the fibers on a seed are not of equal length, there being always, in fact, considerable variation. It was therefore desirable to ascertain the actual lengths in samples representing each of the populations. This was done with the Baer sorter, an instrument by means of which the lint in a sample can be divided into a series of subsamples according to length. The relation each subsample bears to the whole sample is determined and is expressed in percentages. It is then a simple matter to draw a cumulative curve showing the percentages of the various lengths of fiber in the sample. Such curves, based on samples representing each of the three populations, are shown in Figure 4. In interpreting these curves it should be understood that each successive figure on the abscissa from left to right indicates the percentage of the total sample which is of the length indicated, or longer. Taking as an

example the curve for Durango × Pima, it is seen that somewhat more than 40 per cent of the sample is 1¼ inches or longer and that 70 per cent of the sample is 1⅛ inches or longer.

The samples representing the several pollinations were all taken from the same Durango plant. Each sample consisted of the lint from a single boll which had developed from a flower pollinated on August 12 or 13. The lint lengths in the samples representing the several pollinations ranged as follows:

Durango × Pima	0. 45–1. 40 inches	(11. 4–35. 5 mm.)
Durango × Durango	0. 25–1. 35 inches	(6. 3–34. 3 mm.)
Durango × Hopi	0. 15–1. 35 inches	(3. 8–34. 3 mm.)

The longest fibers in the three samples differed little in length, but the shortest fibers from the fertilization Durango × Pima were three times as long as the shortest fibers from the fertilization Durango × Hopi. The shortest fibers from the Hopi fertilization were only three-fifths as long as the shortest from fertilization with pollen of the same variety (Durango), and were, in fact, almost short enough to be classed as fuzz hairs or linters. Since Hopi plants which supplied the pollen, did not show these very short hairs, and since they did not appear on the Durango seeds resulting from fertilization with like pollen, a metaxenia effect not foreshadowed in the expressed characters of either parent is evident in this case.

Lint length was not significantly correlated with any other character determined in the three populations, as is evident from the data given in Table 4. The apparently significant positive correlation with seed weight in the Durango × Hopi population was due wholly to the inclusion of five stunted bolls on a single plant, which contained exceptionally few and light seeds with abnormally short lint, grading only 1.0 to 2.0 as compared with a mean grade of 4.6 in this population. The evidence, therefore, warrants the conclusion that a metaxenia effect on this character has been demonstrated.

INFLUENCE OF POLLEN ON LINT INDEX

The amount of lint on the seed is indicated by the lint index, or weight of lint per seed. In the present investigation, this value was determined for each of the 100 bolls from each of the three pollinations by dividing the total weight of lint in each boll by the number of seeds therein and multiplying the quotient by 100. The three parental families bear a relation with respect to lint index different from that with respect to boll period and lint length, Durango averaging highest (lint index, 6.29), Hopi lowest (lint index, 1.92), and Pima occupying an intermediate position (lint index, 5.25). The relative abundance of the lint in the three families is fairly well shown in Figure 2.

The differences among the mean lint indices of the populations resulting from the several pollinations as given in Table 3 are scarcely significant, although the mean for Durango × Pima exceeds the mean for Durango × Hopi by an amount that is 2.9 times the probable error of the difference. The difference between the mean indices of the populations Durango × Pima and Durango × Durango is 2.4 times its probable error. If the latter difference had been significant, the position would be an interesting one, indicating an increase in the abundance of lint on the Durango seeds as a result of fertilization by pollen of Pima, a variety having a somewhat lower lint index.

On the other hand, fertilization by pollen of a variety having a much lower lint index (Durango × Hopi) did not cause an appreciable decrease in the lint index as compared with that obtained from fertilization with like pollen (Durango × Durango). Hence, even if it could be concluded that this character was influenced by the kind of pollen, the effect did not parallel the parental relations as in the case of boll period, lint length, and seed fuzziness. The analogy is rather with the phenomenon of heterosis, or hybrid vigor, which is usually greater the wider the cross, since fertilization by pollen of Pima, a very distinct species, increased the lint index of Durango, while fertilization by pollen of Hopi, a much more nearly allied species, had no appreciable effect.

It seems unlikely, at first glance, that heterosis could manifest itself in the abundance of the lint hairs which are outgrowths of the epidermis of the seed coat and therefore belong to the body of the mother plant. Evidence was given on a preceding page, however, that heterosis in cotton is shown in increased weight of the seeds on the mother plant. It is probable that the increase is mainly in the weight of the embryo or young daughter plant; but if, as seems likely, increase in weight of the embryo is accompanied by an increase in its volume, a corresponding increase in the area of the seed coat would be a physiological necessity. If increase in the area of the outer seed coat is accompanied by an increase in the number of epidermal cells, an increase in the number of lint hairs, and consequently in the lint index, would be expected.

Reference to Table 3 shows that both the lint index and the mean weight of the seeds were highest in the population Durango × Pima.[6] The coefficients of correlation in Table 4 show that these characters were positively and significantly correlated in all three populations. It therefore seems possible that the observed differences in abundance of the lint, assuming them to be significant, are an indirect manifestation of heterosis, rather than of metaxenia in the strictest sense. It is interesting that heterosis was shown in seed weight and possibly in lint index in the cross-fertilization Durango × Pima but not in the cross-fertilization Durango × Hopi. The former represents a cross between species belonging to very different groups, Pima Egyptian representing the South American tree cotton group and Durango upland the Mexican-Central American group. Hopi cotton also belongs to the latter group, so the cross Durango × Hopi is between much more closely related cottons than the cross Durango × Pima and would be expected to show heterosis in a smaller degree, if at all.[7]

The negative correlations between weight and number of the seeds, highest in the population Durango × Pima, as shown in Table 4, should be considered also in this connection. The mean number of seeds per boll was significantly lower in the Durango × Pima population than in the others (Table 3), so that the greater weight of the Durango × Pima seeds, in part at least, may have been due to better nutrition because of their smaller number. It is impossible to decide the relative importance of the two factors, heterosis and nutrition, in bringing

[6] In mean weight of seeds the differences were highly significant, Durango×Pima having exceeded Durango×Durango and Durango×Hopi by amounts equal, respectively, to six and seven times the probable error of the difference.

[7] Populations representing F_1 and F_2 of the crosses Pima×Hopi and Acala upland×Hopi were compared at Sacaton in 1928 and 1929. The former cross showed much more pronounced hybrid vigor in the F_1 plant and much greater diversity in the F_2 than the latter cross, in which these manifestations were scarcely more pronounced than in crosses between two varieties of upland cotton.

about the greater weight and presumably greater volume of the seeds in this population, but the possibility that the observed difference in lint index is a function of the difference in weight of the seeds is not affected by this uncertainty.[8]

It might be thought that the same line of reasoning would apply also to seed fuzziness, since the fuzz, like the lint, consists of simple outgrowths of the cells of the outer epidermis of the seed coat. In other words, if heterosis manifests itself in an increase of the lint index, it would be expected to manifest itself in increased fuzziness of the seeds. But it was observed in the experiment of 1928 (Table 1) that the seeds on the Pima plants in bolls resulting from cross-fertilization with pollen of another species, Hopi, were both significantly heavier and significantly less fuzzy than in bolls resulting from fertilization with Pima pollen. On the other hand, no significant difference in lint index was detected in this experiment, and lint index and seed fuzziness were negatively correlated in both populations, significantly so in Pima × Pima.[9] (Table 2.) A possible explanation of the absence of correlation is that, while lint abundance was determined by weight, seed fuzziness was determined by grades based not on the weight of the fuzz hairs but on the relation of the fuzz-covered portion of the seed coat to its total area.

Comparison of the coefficients of variation in Table 3 shows that there was less variation in lint index in the population from the fertilization Durango × Durango than in either of the populations from fertilizations with unlike pollen. Each of the differences, C of Durango × Pima minus C of Durango × Durango and C of Durango × Hopi minus C of Durango × Durango, is practically three times its probable error. Here again the position is different from that observed in respect to boll period and lint length, fertilization with Hopi pollen not having resulted in greater variation in lint index than was induced by fertilization with Pima pollen.

EXPERIMENT IN 1930

Acala is the only variety of upland cotton grown extensively in those parts of Arizona where Pima Egyptian cotton is grown, and it is therefore of particular interest to determine whether there are immediate effects of pollen resulting from cross-fertilization between these varieties. An experiment was performed at the United States Field Station, at Sacaton, Ariz., in 1930 to determine whether metaxenia occurs when Pima flowers receive Acala pollen and vice versa.

MATERIAL AND METHODS

Strictly inbred families containing the required number of plants were not available for this experiment, so plants grown from "bulk" seed were used, excluding any that were not typical of the respective variety. Selection was made of 54 plants of each variety, which were numbered consecutively. All bolls that had set previously on these

[8] Significant negative correlations between the number and the mean weight of the seeds in individual bolls of Pima cotton at Sacaton, Ariz., were detected by Kearney in populations grown in 1925, 1927, and 1928, the coefficients having ranged from -0.297 ± 0.043 to -0.413 ± 0.037. An obvious physiological relation is involved in this tendency for fewer seeds to be better nourished and consequently heavier than more numerous seeds.

[9] There seems, however, to be no general association of these characters, for Kearney, who determined the correlation in four Pima populations, each grown in a different year, obtained in only one population a coefficient that was apparently significant, having been 3.2 times its probable error. In this case also the correlation was negative.

plants were removed before the experiment was begun. This was done because it has been found that removal of the bolls produced by the earlier flowers materially reduces boll-shedding from the later flowers (*1*).

The pollinations were made from August 19 to August 28, inclusive. As in previous experiments, all flowers were emasculated the preceding afternoon and pollinated between 10 a. m. and noon of the morning of anthesis. In order to insure ample material, 50 flowers on the Acala plants and 30 flowers on the Pima plants were emasculated and pollinated daily. During the 10-day period of the experiment the desired quotas were obtained on all but one or two days. On the plants of each variety one-half of the flowers were pollinated with their own kind of pollen and the remainder were pollinated with pollen of the other type. The total numbers of flowers pollinated were as follows: Acala × Acala, 243; Acala × Pima, 243; Pima × Pima, 147; Pima × Acala, 147.

Since shedding was less than was anticipated, more material was available at the end of the season than could be handled readily, and it was decided to use only 100 bolls from each pollination of each variety. In order to avoid selection, the bolls, when mature, were collected as follows: Beginning with Pima plant No. 1 and with Acala plant No. 1, all bolls from each pollination were collected, and this was repeated on the successively numbered plants until the desired number of bolls had been obtained. Since on every plant the numbers of bolls from the two pollinations were approximately the same, the individual plants of each variety contributed nearly equal numbers of bolls to the lots representing the two pollinations. Any differences that may have existed among the individual plants were equalized by this method of collection.

The procedure was varied in the case of the Acala plants by collecting separately and in equal number bolls so placed as to be fully exposed to sunlight and bolls so placed as to be shaded during most of the day. This was done in order to ascertain the effect of such difference in positions on the boll period and the length of the lint. Since the plants were spaced approximately 12 inches apart in the row, they had made rather heavy vegetative growth, and as a result almost equal numbers of bolls on all of them were exposed and shaded, respectively. Fifty bolls were collected, representing each subpopulation of the Acala variety, as follows: Acala × Pima, exposed; Acala × Pima, shaded; Acala × Acala, exposed; Acala × Acala, shaded.

The characters determined on the material from this experiment were boll period (number of days from anthesis to opening of the boll) and lint length in both varieties and seed fuzziness in the Pima variety. Since all seeds produced on the Acala plants were entirely covered with fuzz, it proved impracticable to determine differences in respect to the last character in that variety. The determinations were made in the same manner as in the experiments of 1928 and 1929. The statistical constants for the several populations are given in Table 5. The table is arranged so that the upper portion contrasts the effects of the two kinds of pollen in each variety and the lower section contrasts the effects of a fully exposed and of a shaded position of the boll in the Acala variety.

TABLE 5.—*Statistical constants for characters determined on bolls from emasculated flowers of Pima Egyptian and Acala upland cottons, fertilized with pollen of the same and of the other variety*

Character and population	Determinations	Mean	Standard deviation	Coefficient of variation
With reference to pollination:				
Boll period—	*Number*	*Days*	*Days*	
Pima×Pima	100	74.9±0.20	2.98	4.0±0.19
Pima×Acala	100	73.8± .22	3.31	4.5± .21
Difference		1.1± .30		.5± .28
Acala×Pima	100	69.3± .40	5.89	8.5± .40
Acala×Acala	100	66.6± .30	4.52	6.8± .32
Difference		2.7± .50		1.7± .51
Lint length—		*Grade*	*Grade*	
Pima×Pima	100	8.5± .02	.30	3.5± .17
Pima×Acala	100	7.8± .02	.35	4.5± .21
Difference		.7± .03		1.0± .27
Acala×Pima	100	5.0± .03	.40	8.0± .38
Acala×Acala	100	4.4± .03	.39	8.9± .42
Difference		.6± .04		.9± .57
Seed fuzziness—				
Pima×Pima	100	5.8± .08	1.12	19.3± .92
Pima×Acala	100	6.2± .07	.97	15.6± .74
Difference		.4± .11		3.7±1.18
With reference to exposure:				
Boll period—		*Days*	*Days*	
Acala×Pima, shaded	50	72.7±0.37	3.85	5.3± .36
Acala×Pima, exposed	50	66.0± .54	5.69	8.6± .58
Difference		6.7± .65		3.3± .68
Acala×Acala, shaded	50	69.2± .34	3.56	5.1± .34
Acala×Acala, exposed	50	63.9± .36	3.78	5.1± .34
Difference		5.3± .50		0± .48
Lint length—		*Grade*	*Grade*	
Acala×Pima, shaded	50	5.1± .04	.40	7.8± .53
Acala×Pima, exposed	50	5.0± .04	.39	7.8± .53
Difference		.1± .06		0± .75
Acala×Acala, shaded	50	4.5± .04	.44	9.8± .66
Acala×Acala, exposed	50	4.4± .03	.33	7.5± .51
Difference		.1± .05		2.3± .83

INFLUENCE OF POLLEN ON BOLL PERIOD

The boll period, or number of days from anthesis to maturation, is considerably longer in Pima Egyptian than in Acala upland cotton. Data given by King, Loomis, and Varmette (*6, p. 948*) show that at Sacaton in 1923 the difference averaged 7.5 ± 1.7 days for flowers opening during the period August 12 to 16 and 7.8 ± 0.9 for flowers opening during the period August 27 to 31.[10] From the results of the experiments in 1928 and 1929 it would be expected that the flowers fertilized with Pima pollen would have a somewhat longer boll period than the flowers fertilized with Acala pollen. Such proved to be the case, the data in Table 5 showing that fertilization

[10] Data for the intervening 10 days were not obtained by these investigators, but the two periods of 5 days each mentioned above comprise the period (Aug. 19 to 28) during which the flowers used in the present experiment reached anthesis.

of Pima flowers with Acala pollen shortened the boll period by an average of 1.1 days and that fertilization of Acala flowers with Pima pollen lengthened the boll period by an average of 2.7 days. In both cases the differences, in comparison with the result from fertilization with like pollen, were significant, having been, respectively, 3.7 and 5.4 times the probable error of the difference.

Reference to the lower portion of Table 5 shows that the bolls on the Acala plants which were in shaded positions required five or six days longer to mature than the bolls which were exposed to full sunlight during most of the day. The differences between the means for exposed and shaded bolls from each pollination, Acala × Pima and Acala × Acala, are very significant, being in each case about 10 times the respective probable error. It seems unlikely, however, that this difference in length of the maturation period, resulting from different positions of the bolls, influenced materially the mean difference of 2.7 days caused by the kind of pollen with which the Acala flowers were fertilized, for while shading prolonged the mean boll period 6.7 days in the case of Acala flowers fertilized with Pima pollen and only 5.3 days in the case of Acala flowers fertilized with Acala pollen, the difference (1.4 ± 0.8 days) is not significant.

The pollinations in this experiment were completed within a period of 10 days, so there could have been little variation in the conditions as to length of day, temperature, etc., under which the bolls developed. Yet comparison of the coefficients of variation in Table 5 with those in Table 3 shows that while there was less variation in the boll period on the Pima plants in the 1930 experiment than in the boll period on the Durango plants in the 1929 experiment, the latter showed less variation than the boll period on the Acala plants in the 1930 experiment.

INFLUENCE OF POLLEN ON LINT LENGTH

There is a great difference between these cottons in average length of the lint, that of Pima averaging about 1⅝ inches (41.3 mm.) and that of Acala averaging about 1⅛ inches (28.6 mm.). From the results of the experiments of 1928 and 1929, it would therefore be expected that fertilization of Pima flowers with Acala pollen would tend to shorten the lint and that the converse effect would result from the reciprocal fertilization (Acala × Pima). This expectation was fully realized, as is shown by the data on lint length in Table 5. In comparison with the results from fertilization with like pollen (Pima × Pima and Acala × Acala), the lint in the Pima bolls from fertilization with Acala pollen averaged shorter by 0.7 grade (approximately 2.2 mm., or three thirty-seconds of an inch), and the lint in the Acala bolls from fertilization with Pima pollen averaged longer by 0.6 grade (approximately 1.9 mm., or more than one-sixteenth of an inch). The differences in both cases are highly significant, being 23 and 15 times the respective probable error.

No significant difference in length of the lint from Acala bolls that were shaded and from those that were fully exposed to the sun is indicated by the data in the lower portion of Table 5.

The coefficients of variation of lint given in Table 5 indicate no important difference in the amount of variation induced by the two kinds of pollens when applied to flowers of either variety. On the other hand, as in the case of boll period, there was much more variation in both populations of Acala bolls than in either population of Pima bolls.

INFLUENCE OF POLLEN ON SEED FUZZINESS

The seeds of Pima cotton vary considerably in degree of fuzziness, ranging from nearly naked to entirely fuzzy. Both of these extremes are rare, however, the average condition being with about half of the seed coat covered with fuzz. In the experiment of 1928, fertilization of Pima flowers with pollen of a smoother-seeded type, Hopi, resulted in a significant decrease in the average fuzziness. As is shown by the data in Table 5, the fuzziness of the Pima seeds was increased when Pima flowers were fertilized with pollen of the much fuzzier-seeded Acala cotton. The mean difference, in comparison with seeds from fertilization with like pollen (Pima × Pima) is less than one-half grade, but apparently significant, being 3.6 times its probable error. As has been mentioned, differences in respect to this character could not be detected in the seeds from the several populations of Acala bolls.

DISCUSSION

It has been suggested from time to time that fertilization with different kinds of pollen may affect not only the embryo in the seed, which is the young daughter plant, but also other parts of the seed and even the fruit enveloping it, which belong to the body of the mother plant. But it remained for Swingle (*9*) and Nixon (*7*, *8*) to bring forward convincing evidence that such effects occur in the date palm. They found differences in the length of time required for ripening of the fruit, in size of the fruit, and in size and shape of the seed, depending upon the kind of pollen with which fertilization was effected. The differences were most pronounced when female flowers of the date palm were fertilized with pollen of other species of Phoenix. Tissues outside the embryo and endosperm were affected, so the results could not be attributed to heterosis or to xenia, the well-known phenomenon in maize which has been accounted for as due to fertilization of the endosperm by the second generative nucleus of the pollen tube. The term "metaxenia" was invented by Swingle to designate pollen effects on the somatic tissues of the mother plant.

Evidence is presented in this paper that metaxenia occurs also in cotton, and the fact of its occurrence in the wholly unrelated groups Phoenix and Gossypium makes it probable that the phenomenon is of widespread occurrence among plants. The characters of the cotton plant in which metaxenia effects were most clearly shown—length of the lint hairs and quantity of fuzz—are both properties of the seed coat, which belongs to the body of the mother plant. The time required for development of the boll also was influenced by the kind of pollen, but in much less degree than was the time required for maturation of the date fruits in Nixon's experiments.

The metaxenia effects so far discovered in cotton parallel closely the parental relations of the characters. Thus pollen of the quickly maturing, short-linted, smooth-seeded Hopi cotton, when applied to flowers of the slowly maturing, long-linted, relatively fuzzy-seeded Pima cotton, reduced the length of the boll period, shortened the lint 7.5 per cent, and reduced the fuzz on the seeds, although the effect on the first character apparently was not significant. Conversely, pollen of the slowly maturing, long-linted Pima cotton, applied to flowers of the more rapidly maturing and shorter-linted Durango and Acala cottons, significantly increased the length of the

boll period and the length of the lint, the increase in lint length amounting to 8.2 per cent in the cross-fertilization Durango × Pima and to 13.5 per cent in the cross-fertilization Acala × Pima. Finally, pollen of the more rapidly maturing, shorter-linted Acala cotton, applied to flowers of Pima cotton, significantly shortened the boll period and decreased the length of the lint, the decrease in lint length amounting to 8.2 per cent.

All of these cross-fertilizations are between species belonging to very distinct groups, Pima representing the South American group, which comprises the sea-island and Egyptian cottons, while Hopi, Durango, and Acala belong to the Mexican-Central American group, which comprises the upland cottons and related forms. Cross fertilizations between two members of the latter group, Durango and Hopi, did not significantly affect the time required for development of the boll, but the length of the lint on the Durango plants was reduced significantly to the extent of 6.1 per cent by fertilization with pollen of the shorter-linted Hopi cotton.

Heterosis, or hybrid vigor, was found to be much greater in the wide crosses Pima × Hopi, Durango × Pima, and Pima × Acala than in the cross between species of the same group, Durango and Hopi. The data are too scanty, however, to permit the conclusion that in cotton metaxenia effects also are greater the wider the cross. Nixon found this to be the case in Phoenix, for when flowers of the date palm (*P. dactylifera*) were fertilized with pollen of other species (*P. canariensis*, *P. roebellenii*), a more pronounced degree of metaxenia resulted than from cross fertilizations among different date palms.

The effects in these interspecies fertilizations in the genus Phoenix, unlike those in the interspecies fertilizations in Gossypium, were not predictable from the characters of the male parent. Thus fertilization of flowers of *Phoenix dactylifera* with pollen of *P. canariensis*, which has a very large, blunt seed, resulted in smaller and more pointed seeds than are produced by the fertilization *dactylifera* × *dactylifera*, and the seeds resulting from the cross fertilization *dactylifera* × *roebellenii* showed characters not seen in either of the parent species. The only analogy to the last-named effect discovered in cotton was the appearance on Durango seeds resulting from fertilization with Hopi pollen of very short lint hairs, shorter than were found on the seeds of either parent.

The nature of the mechanism involved in these metaxenia effects is unknown. Swingle and Nixon have surmised that there is an analogy to hormone action in the bodies of animals, the hypothesis being stated by Swingle (*9, p. 266*) as follows:

> The simplest and most probable theory to explain metaxenia is that the embryo or endosperm or both of them secrete hormones, or soluble substances analogous to them, which diffuse out into the tissues of the mother plant that constitute the seed and fruit and there exert a specific effect on these tissues, varying according to the particular male parent used to fecundate the embryo and endosperm.

Since in the seeds of cotton the endosperm is but slightly developed and is absorbed at a relatively early stage, it seems likely that if substances analogous to hormones are produced they are secretions of the embryo rather than of the endosperm.

The parallelism in cotton between the metaxenia effects and the characters of the pollen parent suggests the hypothesis that secretions

may be initiated by the genes which determine the characters, but the absence of such parallelism in Phoenix is an obstacle to the generalization of this idea.

The demonstration of metaxenia in cotton is of practical interest in relation to uniformity of lint length, a very important property. It has been shown that fertilization of Pima cotton flowers with pollen of the shorter-linted Hopi and Acala cottons shortened the lint on the Pima plants by more than one-sixteenth of an inch as compared with the lint produced by flowers fertilized with Pima pollen. Fertilization of Durango and of Acala upland flowers with pollen of the long-linted Pima cotton, on the other hand, lengthened the lint approximately one-sixteenth of an inch as compared with the lint from flowers fertilized with like pollen. Such differences in length are not negligible from the spinner's point of view.

Wherever two varieties of cotton are grown in neighboring fields, more or less transfer of pollen by bees and other insects occurs. Only a small percentage of the ovules in a field are likely to be cross-fertilized in this way, but if the two varieties differ greatly in staple such crossing as takes place will be detrimental to the uniformity of the immediate product as well as to the value of the seed for future planting. A certain proportion of the lint will be shorter than the average in the field of long-staple cotton, and longer than the average in the field of short-staple cotton. The discovery of metaxenia in respect to lint length therefore gives an additional reason for advocating the growing of only one kind of cotton in a neighborhood.

SUMMARY

Experiments to determine whether metaxenia, or immediate effects of pollen on tissues of the mother plant, occurs in cotton were suggested by the discovery of this phenomenon in the date palm by Swingle and Nixon.

The first experiment involved two different types of cotton, Pima Egyptian and Hopi. These forms represent the two main groups of New-World cottons, the Pima belonging to the South American group, and Hopi to the Mexican-Central American group.

Flowers on some of the Pima plants were fertilized with Hopi pollen, while flowers on other Pima plants were left to natural pollination.

The boll period, or number of days from anthesis to opening of the boll, was computed from a record of each individual boll. Determinations of length of lint, lint index, seed fuzziness, number of seeds per boll, and weight of the individual seeds were made on approximately 100 bolls from each fertilization. The statistical constants of the six characters are given in Table 1, and the coefficients of correlation among them (excepting boll period) are given in Table 2.

A mean difference of 18 days between the boll periods of the parent types was observed. The boll period on Pima plants averaged 1.1 days shorter from flowers fertilized with Hopi pollen than from flowers fertilized with Pima pollen. Environment is believed to have neutralized partly the effects of unlike pollen.

The lint lengths (fig. 2) of Pima and Hopi average about 1⅝ inches and seven-eighths of an inch, respectively. A significant reduction in length of lint of a full one-sixteenth of an inch resulted from fertilization of Pima flowers with Hopi pollen. Consideration of the cor-

relations of lint length with the other characters studied makes metaxenia a reasonable explanation of this difference.

Pima and Hopi differ greatly in abundance of lint on the seeds (fig. 2), but the means for lint index of seeds on Pima plants resulting from fertilization with Pima and with Hopi pollens, respectively, did not differ significantly.

When flowers of the relatively fuzzy-seeded Pima family were fertilized with pollen from the nearly naked-seeded Hopi family (fig. 3), a significant reduction in fuzziness resulted. Correlations of seed fuzziness with other characters in these populations do not invalidate the conclusion that this difference also is a metaxenia effect.

The mean weight of the individual seed from the fertilization Pima × Hopi was significantly greater than from the cross-fertilization Pima × Pima, but this difference is attributed to heterosis rather than to metaxenia.

A second experiment involved Pima Egyptian, Durango upland, and Hopi. The length of lint of Pima averages 1⅝ inches, of Durango 1¼ inches, and of Hopi seven-eighths of an inch. (Fig. 2.) The boll period of Durango averages longer than that of Hopi but much shorter than that of Pima.

Durango, because of its intermediate lint length, was used as the female parent. Approximately equal numbers of flowers were pollinated daily with Pima, Durango, and Hopi pollens. A rotation was followed that resulted in approximately equal numbers of bolls from each of the three fertilizations on each of the plants and equalized individual plant differences and seasonal variations.

The characters studied were the same as in the first experiment except seed fuzziness, which was disregarded because of the extreme fuzziness of Durango seeds. (Fig. 3.) The statistical constants of the characters determined on 100 bolls from each fertilization are given in Table 3, and the coefficients of correlation among them are given in Table 4.

The mean boll period on Durango plants was slightly but significantly increased by fertilization with Pima pollen as compared with fertilization by Durango and by Hopi pollen. Since there was no correlation of boll period with other characters, a metaxenia effect is clearly shown.

Pollen of the longer-linted Pima variety lengthened the lint of the intermediate Durango variety, and pollen of the shorter-linted Hopi variety shortened it. The absence of correlation of lint length with other characters determined leaves metaxenia as the only satisfactory explanation of the differences observed.

Very short lint hairs (fig. 4) on Durango seeds resulting from fertilization with Hopi pollen was the only extra-parental character showing analogy to those discovered by Nixon in cross-fertilizations between different species of Phoenix.

Fertilization of Durango flowers with Pima pollen resulted in a higher mean lint index than fertilization with Durango and with Hopi pollens. The differences are scarcely significant and are probably attributable to heterosis, since lint index was positively correlated with seed weight in all three populations.

A heterosis effect on weight of seeds analogous to that shown by the F_1 plants of hybrids between upland and Egyptian was observed in the cross-fertilization Durango × Pima as compared with the cross-

fertilization Durango × Durango. Cross-fertilization between the closely related forms Durango and Hopi did not affect weight of seeds.

Almost as great a metaxenia effect on lint length of Durango upland cotton was produced by Hopi pollen as by Pima pollen, although the hybrid plants show pronounced heterosis even in the embryonic stage in crosses between upland and Pima cottons and very little, or none, in crosses between upland and Hopi cottons.

Reciprocal cross-pollinations of Pima Egyptian and Acala upland cottons, the varieties most extensively grown in the Salt River Valley, Ariz., gave results in agreement with those of the earlier experiments. The time required for maturation of the boll was significantly decreased when Pima flowers were fertilized with Acala pollen and was significantly increased when Acala flowers were fertilized with Pima pollen. The lint length was significantly decreased when Pima flowers were fertilized with Acala pollen and significantly increased when Acala flowers were fertilized with Pima pollen. In both cases the difference, in comparison with the lint length from fertilization with like pollen, averaged slightly more than one-sixteenth of an inch. The fuzziness of the seeds on the Pima plants was slightly but significantly greater when the flowers were fertilized with Acala pollen.

The metaxenia effects in cotton have been observed in the length of time required for the fruit to mature, in length of lint, and in quantity of fuzz on the seeds. The effect on the first character has been observed in a much more pronounced degree in the date palm. Lint length and fuzziness are properties of the outer seed coat, which is part of the body of the mother plant.

Contrary to the discoveries by Nixon in the genus Phoenix, the metaxenia effects disclosed in cross-fertilizations between different species of cotton parallel the expression of the characters in the parent species.

The theory advanced by Swingle, which attributes metaxenia in Phoenix to a hormonelike action of substances secreted by the embryo or the endosperm, seems to account equally well for the results obtained in Gossypium.

The metaxenia effect on length of lint of cotton suggests the danger of growing two or more varieties of widely divergent staple lengths in the same vicinity, as the uniformity of both products is likely to be impaired to the extent that cross-fertilization occurs.

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DEVELOPMENT OF THE CITRUS-SCAB ORGANISM, SPHACELOMA FAWCETTII[1]

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INTRODUCTION

During February and the spring of 1925, field observations were made by the writer on characteristics of the citrus-scab organism (*Sphaceloma fawcettii* Jenkins) (*9*) [3] as developed on citrus in the rutaceous collection at the United States Citrus Disease Field Laboratory at Orlando, Fla. Based on these studies, the general aspect of the disease, particularly as affecting leaves of citrus, was later outlined by the writer, as reported by Fawcett and Lee (*7, p. 223–226*,[4] *487, and fig. 63*).

In September, 1928, additional observations were made in the same place, as well as in the collection at the Florida Agricultural Experiment Station at Gainesville. The present paper embodies the data thus obtained, especially those based upon the superficial development of the fungus in Florida on spring and autumn growth of leaves of sour orange (*Citrus aurantium* L.) and on young fruit of grapefruit (*C. grandis* Osbeck), as shown in Plate 1 and Figures 1 and 2, and on similar leaf growth of grapefruit and Tahiti lime (*C. aurantifolia* Sw.). Secondary fungi associated with the fungus are mentioned, and data on cultural studies and inoculation tests are included.

The main purpose of the present paper is to present data relative to citrus scab and its pathogene that will aid in distinguishing them from other diseases and organisms with which they have been confused. For this reason data such as those pertaining to the coloration of the fungus are given in considerable detail. Except in the historical section, no reference is made to scab in other citrus-growing countries, as the writer hopes to present later an account of comparative studies of the pathogene in this and other countries.

HISTORICAL REVIEW

The first published report of the occurrence of *Sphaceloma fawcettii* in Florida, in other citrus-growing regions of the United States, or

[1] Received for publication Nov. 25, 1930; issued May, 1931. Most of the data presented in this paper, of which the title was previously published (*11*)[3] as "Certain Characteristics of the Citrus-Scab Organism," are part of a thesis presented to the faculty of the Graduate School of Cornell University in June, 1927, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Thanks are due H. M. Fitzpatrick, L. M. Massey, and A. J. Eames, under whose direction the work was done. The writer also appreciates the assistance rendered by H. R. Fulton, F. W. Pennell, T. R. Robinson, H. E. Stevens, G. F. Weber, Erdman West, O. E. F. Winberg, J. R. Winston, and others in obtaining some of the material on which the studies were based, as well as that rendered by H. S. Fawcett in various other ways. The work was done in cooperation with the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and with the Department of Plant Pathology at Cornell University and at the University of Florida. Color drawings are by J. Marion Shull, and photographs and photomicrographs by Marcel L. F. Foubert, with the exception of those in Figure 4, C and D, which are by J. F. Brewer.

[3] Reference is made by number (italic) to Literature Cited, p. 557.

[4] In the account reported by Fawcett and Lee the next to the last sentence concluding the first paragraph should read as follows: "Old lesions on grapefruit often remain pink or rose colored, presenting a smooth scarred appearance."

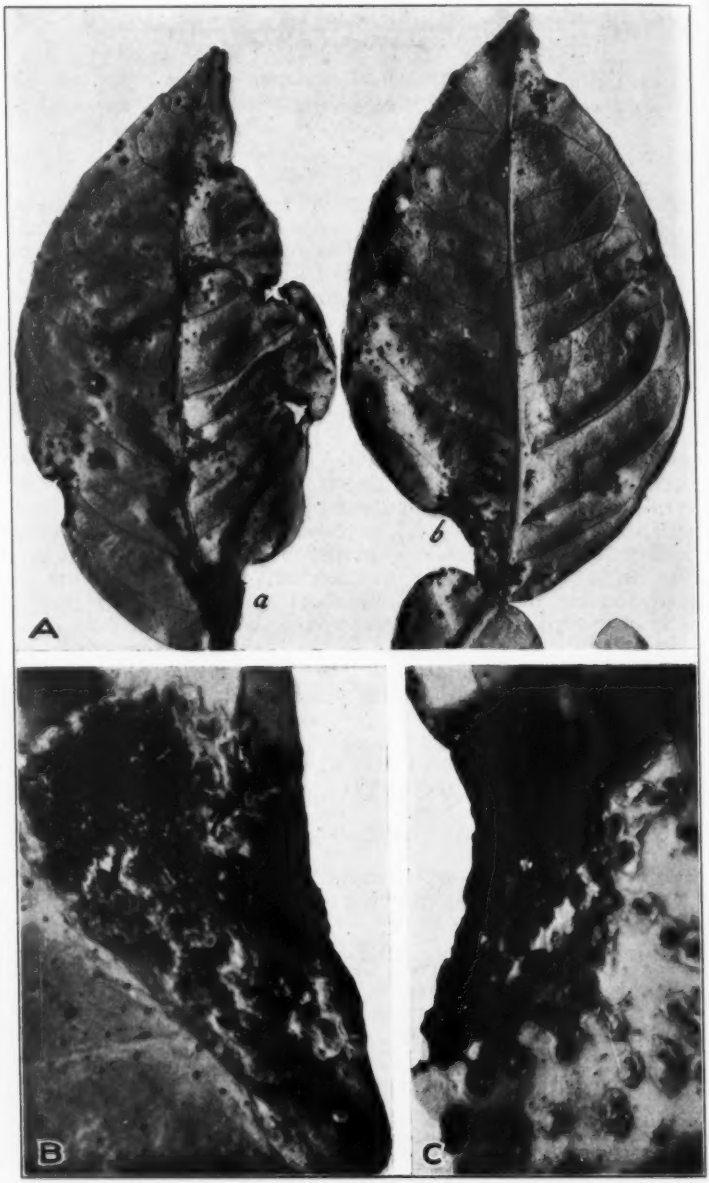

FIGURE 1.—A, Abundant dark conidial fructifications of *Sphaceloma fawcettii* on lower surface of fairly young sour-orange leaves. × 1. B, Enlargement of A, *a*. × 6½. C, Enlargement of A, *b*. × 6½. Material from Gainesville, Fla., September, 1928

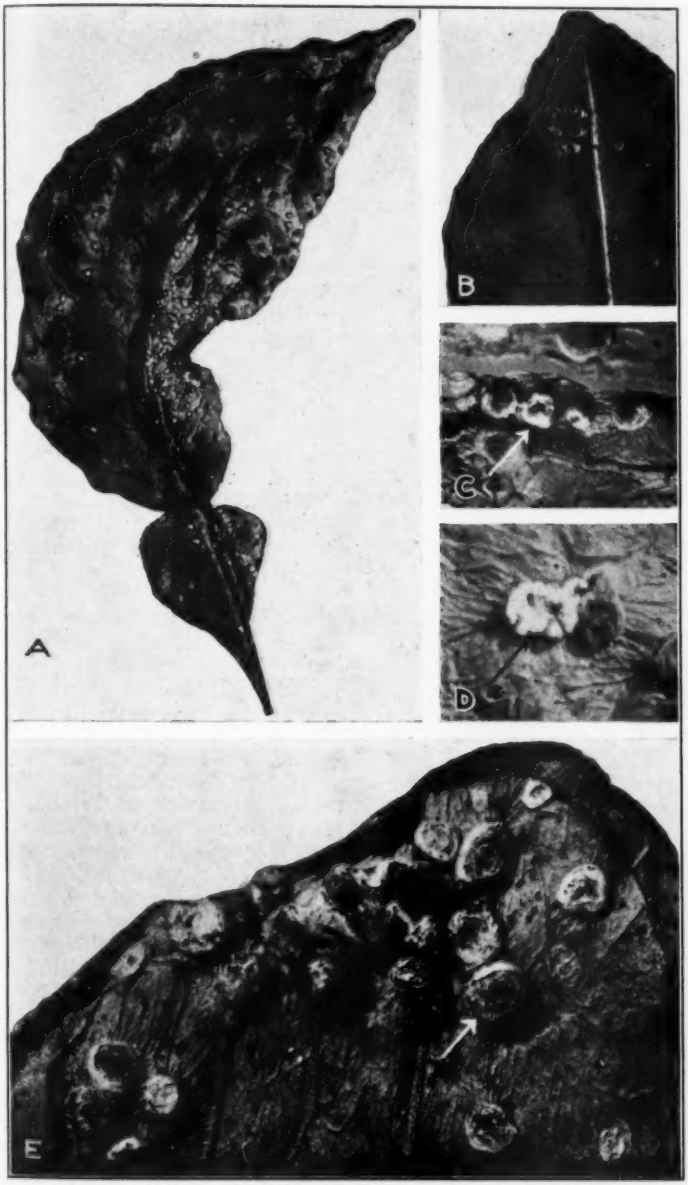

FIGURE 2.—*Sphaceloma fawcettii* on lower side of 2-weeks-old scabbed sour-orange leaves. A, White or pale masses of conidiophores and conidia. × 1. B, *a*, Dark-colored masses of conidiophores and conidia. × 1. C, *a*, and D, *a*, Secondary Fusarium. × 6½. E, Isolated groups of conidial fructifications, some in circular arrangement (*a*) on rims of prominent galls. × 6½. Material from Gainesville, Fla., September, 1928

even in the Western Hemisphere, appears to be that by Scribner (*17*), dated August, 1886. The account is based upon specimens or information transmitted to the United States Department of Agriculture from Ocala and Deland, Fla., during 1885 and 1886. Swingle and Webber (*21*) later noted that the disease had been recognized in Florida "in about the year 1884." W. T. Swingle has recently informed the writer that this date is based upon the observation of scab at that time in a nursery near Lake Weir, afterwards visited by himself and H. J. Webber. From examinations of specimens from Florida and Paraguay recently identified by the writer and now inserted in the Mycological Collections of the Bureau of Plant Industry, it is evident that the organism was established on citrus in both North America and South America at an even earlier date. The specimen from Florida, labeled only "May 1, 1878, Fla.," was originally from a collection of fungi owned by the late W. H. Seaman. The more complete information on the label of the South American specimen reads as follows: "B. Balansa—Pl. du Paraguay. 1878–1884. No. 3543. Ecorces d'oranges douces. Villa Rica, 13 janvier 1882." On this specimen, although acervuli were numerous, no conidia were seen, so that until additional data are available the specific identification of the fungus as *S. fawcettii* should perhaps be regarded as not absolutely positive. The circular arrangement of scab lesions on leaves of citrus (fig. 2, B) grown in Paraguay, noted by Spegazzini (*18*), corresponds to that on such growth developed in Florida and later described by the writer, as reported by Fawcett and Lee (*7, p. 225*).

Scribner (*17*) thought that *Sphaceloma fawcettii* was of recent occurrence in Florida. Swingle and Webber (*21*) expressed the opinion that it had been introduced on Satsuma (*Citrus nobilis unshiu* Sw.) from Japan, from which country the first importation of this citrus variety was brought to Florida in 1876 (*1*). As identified by W. T. Swingle for the writer, the kind of citrus represented by the Seaman specimen is probably sour orange, which is the same species as that on which the Satsuma was first budded in this country (*1*). It is of interest to note that the fungus is not present on material of sour orange and other citrus varieties, not including Satsuma, contained in the Schweinitz herbarium, as acquired by the Academy of Natural Sciences, Philadelphia, in 1834. These specimens bear no date.

Comparisons of *Sphaceloma fawcettii* with the similar species of Sphaceloma causing the disease of avocado (*Persea spp.*) known as avocado scab, made in 1925 by the writer (*10*), revealed that these two organisms had formerly been confused (*19, 20, 25*) and that the one from citrus is not pathogenic on avocado, or at least not on some varieties, as heretofore supposed (*19, 20*). On the basis of information then in part unpublished, the writer (*9*) had formerly assumed that the two organisms or diseases were identical.

Scribner (*17*) reported that sour orange and lemon (*Citrus limonia* Osbeck) were particularly susceptible to citrus scab, but that the sweet orange (*C. sinensis* Osbeck), even now regarded as immune or highly resistant (*25, 26,*) was not affected. Swingle and Webber (*21*) commented upon the economic importance of scab in the lemon groves of Florida, and more recently Winston (*25*) has explained that to this disease was largely due the "failure of the lemon industry in" that State, which before the fungus became established there

"gave promise of becoming a very profitable undertaking." Many rutaceous hosts other than those named in the present paper are known to be susceptible to attack by *Sphaceloma fawcettii*, or by what, so far as known, is this species (*25*, *26*). This fungus, or at least a Sphaceloma on citrus, is firmly established in all citrus-growing regions where climatic conditions favor its development (*6*, *13*, *25*). Peltier and Frederich (*13*) do not include Java among such regions, but scabbed citrus at hand, collected at Buitenzorg in 1919 by R. D. Rands, leaves no doubt of the occurrence there of a Sphaceloma on citrus. Whether this organism is identical with *S. fawcettii* remains to be determined.

FRUCTIFICATIONS ON SCAB LESIONS

DISTRIBUTION

On young scab lesions found on leaves the fungus generally fruits in abundance on the surface originally infected. It was with reference to this surface that the writer made the statement (*7*, *p. 224*) that the central, often craterlike area of young galls (fig. 2, B, *a*), i. e., the type of lesion whose histologic structure is described by Cunningham (*3*) and Butler (*2*), is covered with a fine velvety layer of conidiophores and conidia, which slowly disappears with the aging of the leaf. As concerns young lesions of the gall type, particularly those considerably raised, subsequent observations have revealed that such surfaces from the first may be practically free from fructifications of the fungus; or that the fructifications may occur only in small solitary clumps or larger masses on the marginal regions or rims (fig. 2, E), sometimes outlining troughlike depressions or grooves (figs. 2, E, *a*, and 3, A, *a*). Where a layer of phellem extends as a continuous band from one side of the leaf to the other, the fungus sometimes fruits on both surfaces of the intervening region of the lesion, although in greater abundance on the surface originally infected. Where the necrotic centers of such lesions have fallen away, the fungus may fruit scantily on the faces of the hypertrophied tissue thereby exposed. On lesions in which the tissue has become completely necrotic (fig. 1, A, *b*, and C), possibly owing to the absence of a well-developed phellem, conidial fructifications often cover practically the entire area of the surface originally infected (fig. 3, B), and may also be numerous on the opposite side (fig. 3, D). The necrotic lesion shown in Figure 1, A, *b*, and C, of which a section is shown in Figure 3, D, is a compound lesion; while that illustrated in Figure 3, B, as in the case of the gall type of lesion shown in Figure 3, A, probably resulted from an individual infection. Fructifications of the fungus were present only on the lower side of the compound lesion of the gall type illustrated in Figure 1, A, *a*, and B; that is, on the surface there represented.

At Orlando, Fla., during the spring of 1925, a record was made of the persistence of conidial fructifications of *Sphaceloma fawcettii* on living scabbed leaves of sour orange. The record showed that they may be present on the same lesions for as many as four months.

COLORATION

Masses of conidia and conidiophores on the surface of the lesions on young fresh leaves, stems, and fruits of citrus, as observed at

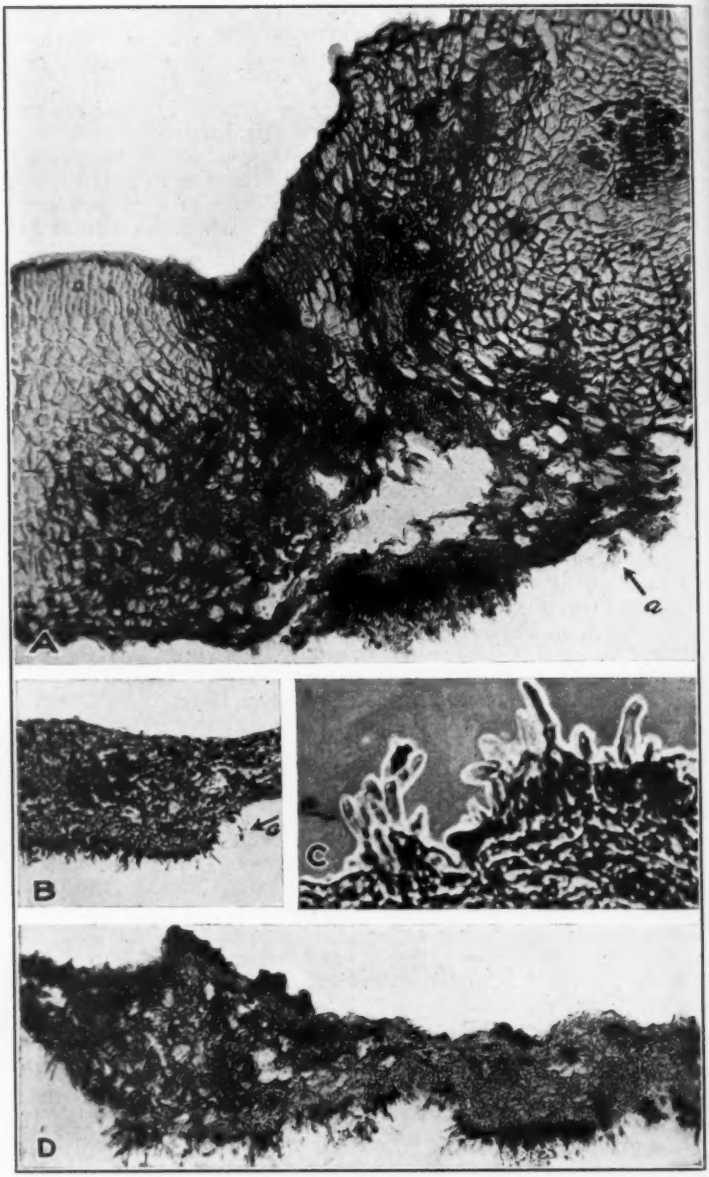

FIGURE 3.—Cross sections through scabbed leaf lesions. A, Large sporodochium on concavity on lower surface of gall; *a*, solitary clump of conidiophores and conidia. × about 200. B, Fructifications covering thickened region of necrotic lesion. C, Enlargement of peripheral region of B, showing more clearly (*a*) clump of conidiophores bearing conidia there labeled *a*. B, × 100; C, × about 600. D, Fructifications on compound necrotic lesion, covering not only the entire lower surface but also part of the upper. × about 200. Material from Gainesville, Fla., September, 1928

A, scab lesions on young fruit of grapefruit; B–D, scab lesions on lower side of several-weeks-old leaves of sour orange, conidial fructifications of the causal fungus visible only on the confluent lesion shown in C. ×1. Material from Orlando, Fla., in the spring of 1925

Orlando during the spring of 1925, were usually drab[5] in color; on leaves approximately 2 months old they ranged from drab to vinaceous drab or dark olive-gray (*7, p. 224–225*).[6] Other colorations of the fungus observed in 1925 are Saccardo's umber, pinkish cinnamon, fuscous, and a brownish color termed "hair brown." Hair brown represented the color of the fungus on a single leaf of grapefruit fully 2 months old, and fuscous its development on succulent leaves not more than 2 weeks old. The older leaf was taken from a large tree and the younger ones from small nursery plants. Of the numerous color readings made at Orlando in 1925, these particular colorings were recorded only once. On young scabbed citrus observed at Orlando in the autumn of 1928 the Sphaceloma cover on the surface of the lesions, as well as the lesions themselves, was of essentially the same appearance as in the spring of 1925. None of the fuscous-colored growth was observed. At Gainesville, however, this was the prevailing color of conidial fructifications of the fungus on young sour-orange leaves (figs. 1 and 2, B and E), although occasionally they were pure white or just becoming colored (fig. 2, A). Contrasting sharply with the warm bright hues of the lesions or the immediately surrounding green healthy leaf surface, the fructifications were most noticeable when fuscous in color. (Figs. 1 and 2, B.) In their drab or nearly drab coloration (pl. 1, C), however, they were rather delicate in appearance and, except as seen through a hand lens, often were practically obscured by the warm hues of the lesions themselves (pl. 1, A, B, and D).

MICROSCOPIC APPEARANCE AND DEVELOPMENT

Conidiophores

Many of the small, compact acervuli of *Sphaceloma fawcettii*, often enlarging to form sporodochia (fig. 3, A and D), were originally confined to a single cell or a few epidermal cells. Their early development was similar to that previously illustrated by the writer (*9, fig. 3*) for this fungus as produced in culture and here represented in Figures 4, A, and 3, C, *a*. The latter illustration shows the formation of conidiophores in the leaf tissue, but whether they are beneath the epidermis or beneath only the cuticle is not clear. At an earlier stage in its development the acervulus shown in Figure 4, A, *b*, probably resembled that represented in Figure 4, A, *c;* both apparently arose from horizontal hyphae like those faintly shown in Figure 4, A, *a*. Original clumps of conidiophores were often distinct, even after continued growth from them had taken place. (Fig. 4, E and G.) As in culture (*9, fig. 3*), when first produced on the citrus substrate the conidiophores were mostly continuous or 1-septate (fig. 4, A, *b*). They were sometimes colored even before being exposed; often they seemed to be firmly held together, or embedded, in a clear hardened substance apparently derived from their walls. Where conidiophores had continued to grow, by the formation of conidia remaining in situ or by further hyphal growth, they were ordinarily not more than 25 or 30 μ long, suggesting those of Cladosporium or Fusicladium. In the unusually large sporodochial development on the gall type of lesion represented in Figure 3, A, the longest conidiophores measured approximately 100 μ. Some of those composing

[5] Ridgway, R. Color standards and color nomenclature. 43 p., illus. Washington, D. C. 1912.
[6] Color readings, based on Ridgway's color guide, are by J. Marion Shull, F. R. Cole, and the writer.

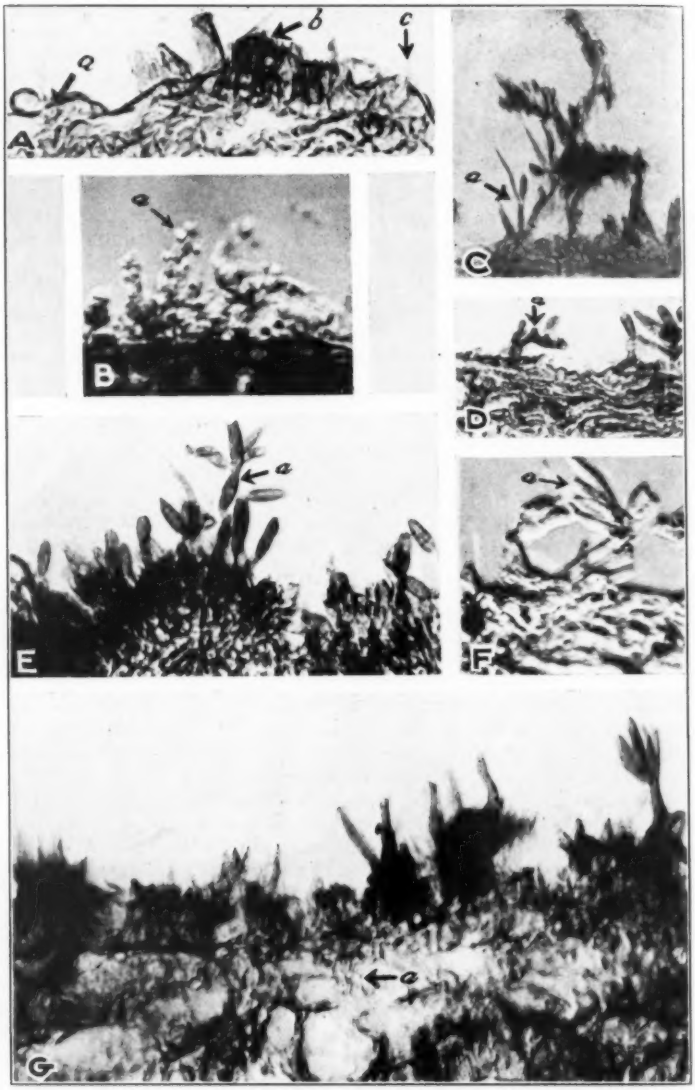

FIGURE 4.—Parts of cross sections through necrotic lesions. A, *a*, Hyaline hyphae; *b* and *c*, acervuli. × about 600. B, *a*, Small spherical conidia produced on conidiophores. × about 600. C, *a*, A conidiophore which first produced a conidium and then continued its growth as a hypha instead of producing another conidium. × 400. D, *a*, Septate elongate conidium which has produced a secondary conidium while still attached to conidiophore. × 400. E, Enlargement of part of section shown in Fig. 3, D; *a*, conidium in situ from which several secondary conidia have developed. × about 600. F, *a*, Group of spindle-shaped conidia partially dislodged from conidiophores. × about 600. G, Small clumps of conidiophores one of which bears a cluster of conidia at its apex; *a*, hyphae penetrating the leaf structure. × about 600. Material from Gainesville, Fla., September, 1928

the pale cottony growth illustrated in Figure 2, A, attained twice this length. Dark conidiophores of more usual length often arose from the entire surface of thick pale-colored stromata, covering the central areas of lesions of the gall type or extending for a considerable distance on the surface of lesions entirely necrotic. Figure 4, C, *a*, represents a conidiophore which first produced a conidium and then continued its growth as a hypha instead of producing another conidium.

Conidial Forms

Conidia in preparations made from the Sphaceloma cover on scab lesions consisted of (1) ovoid elliptical hyaline conidia, (2) ovoid elliptical (fig. 3, C, *a*) or spindle-shaped (fig. 4, F, *a*) colored conidia, and (3) small spherical conidia (fig. 4, B, *a*) not previously reported. These three types were considered to be homologous or merely to represent older or younger forms. The colored conidia ranged from pale yellowish to reddish brown or nearly black. At times it could be discerned that their dark coloration was limited to a hardened outer wall or epispore. Occasionally this was irregularly thickened or roughened, although ordinarily it was smooth. Where the epispore of dark-colored elongate conidia had become ruptured the hyaline endoconidia thus exposed were indistinguishable from newly formed elongate hyaline conidia such as have been previously illustrated by the writer (*9, figs. 3 and 4*).

The elongate colored conidia, which were present in great numbers, commonly measured about 10 by 4 μ, although occasionally they reached 16 μ in length. They were usually continuous but sometimes 1-septate or, more rarely, 2-septate. (Figs. 3, C, *a*, and 4, E–G). In the 1-septate type a number were seen to be breaking apart at the septum or to have become entirely separated. They were sometimes produced on the sides of the conidiophores, or what were originally conidia, all or part of the way from the base to the apex, extending outward more or less at right angles. (Fig. 4, E, *a*.) The septate structure shown in Figure 4, D, *a*, appears to be a primary conidium continuing its growth in situ on the conidiophore on which it was borne; a secondary conidium has been produced.

Elongate hyaline conidia were usually seen in preparations made during periods of precipitation or when the leaves were wet with dew. Usually detached, many of them had sprouted from colored elongate conidia of which they were only younger forms; they were usually produced at the end or perhaps even more commonly at a point just to the side, as is illustrated by Petch (*14, figs. 3 and 4*) for what appears to be a comparable mode of conidial formation in *Myriangium duriaei* Mont. and B., not hitherto interpreted as such. They were formed in the same manner in from 1 to 3 hours when the elongate, colored conidia were sown in drops of water on glass slides.

The spherical microconidia were seen on numerous occasions at Orlando, Fla., during the spring of 1925. They ranged from 1 to 4 μ in diameter. Those of the hyaline type were often glistening or highly refringent, particularly when thick-walled. As in the case of the elongate conidia, they sometimes budded from each other. On a few occasions they were produced from the surfaces of elongate colored conidia sown on corn-meal agar medium. The masses of glistening or refringent bodies embedded in the free mucilaginous

substance previously reported as present in this fungus (*7, p. 490*) were probably masses of microconidia. In one instance small glistening microconidia, about the size of those shown in Figure 4, *a*, were seen grouped about the pointed apexes of conidiophores still contained within an unruptured epidermal cell. Possibly under more favorable circumstances the larger elongate conidia would have formed. Assuming that the refringent granules observed in *Sphaceloma ampelinum* DBy. by Prilleux (*15, p. 316; 16, p. 37–38*), by Viala (*22, p. 304–305, fig. 119*), and by Viala and Pacottet (*23, p. 663; 24, p. 89, fig. 22*) were all microconidia, those of *S. fawcettii* would, of course, correspond to them, as in the case of those produced by *S. symphoricarpi* Barrus and Horsfall (*12*).

The comparatively few swollen or germinated conidia seen on the surface of scab lesions corresponded to those formerly illustrated as developed in culture from elongate hyaline conidia (*9*). Elongate colored conidia that had become greatly swollen and 1-septate or muriform suggested forms referred to Coniothecium by Guéguen (*8, pl. 10*). Spherical hyaline bodies, visible through the walls of one of them, were probably homologous to the sporelike bodies observed in culture by Fawcett (*5*) within what was probably the original cell of germinated hyaline conidia 3 days old. Several hundred small conidia of various sizes, present in the vicinity of an elongate colored conidium that had germinated, apparently had been produced from its surface.

ISOLATIONS AND CULTURES

Sixteen or more isolations of *Sphaceloma fawcettii* from scabbed citrus grown in Florida were made by the writer during 1925 and 1926. Most of them consisted of dilution-plate cultures from the elongate colored conidia on bittersweet orange, sour orange, grapefruit, Satsuma, Tahiti lime, calamondin (*Citrus mitis* Blanco), and a lemon hybrid. A single set of isolation cultures often produced hundreds of small colonies characteristic of those previously described for this organism (*4; 5; 7, p. 490; 25*). In order to compare them in parallel, a culture from practically every set of isolations was preserved. The inoculum for such stock cultures ordinarily consisted of a single isolated colony; in most cases this probably represented growth from a single conidium. One culture from Tahiti lime (culture 71), was definitely known to have developed from a single conidium. These cultures and culture 8, which is the single conidium strain of the Stevens culture isolated in 1916 (*9*), were compared in parallel on test-tube slants of carrot, Molisch's, potato-dextrose, and other agar media. The inoculum here consisted of a mass of stromatic growth about 5 mm. in diameter transferred from each stock culture. In common with culture 8, on the three media named most of the cultures produced a convoluted type of colony. (Fig. 5, A.) Culture 71, however, proved to be decidedly pulvinate as compared with culture 8 or with most of the other isolations that have been made to date. (Fig. 5, B.) Its coloration also was somewhat different; for example, compared in parallel with culture 8, in a certain set of 17-day-old cultures on carrot agar, it was vinaceous brown, ranging lighter and darker; whereas culture 8 was at the same time Tilleul buff. An isolation from bittersweet orange showed features intermediate between those of cultures 8 and 71, in being pulvinate or hemispherical

on Molisch's medium, even when several weeks old (fig. 5, C) and definitely convoluted on carrot-agar medium (fig. 5 D.)

The culture from the bittersweet orange is no longer alive, but cultures 8 and 71 are still in the stock-culture collection. Although they have been transferred many times, their gross cultural characteristics still remain distinct as described. Such marked cultural variations are of course to be taken into consideration when comparisons are based upon gross cultural characteristics. Their significance otherwise is not understood. It may be noted, however, not only that corresponding cultural differences have been observed by the writer for some of the species of Sphaceloma listed in a previous publication (*12, p. 46–47*), but also, judging by Petch's (*14*) illustrations, that similar types of growth occur in Myriangium as developed on the host plant. Fawcett has informed the writer recently that in *Sphaceloma fawcettii* he has observed the pulvinate as well as the convolute type of growth here described.

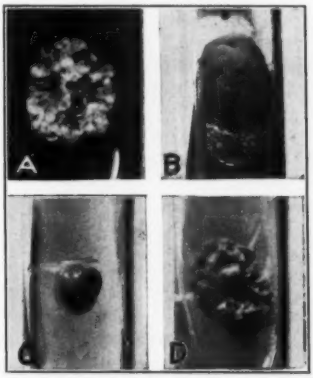

FIGURE 5.—*Sphaceloma fawcettii* on agar media. Convoluted (A) and pulvinate (B) types of colonies represented by two different isolations grown on the same medium, potato-dextrose, and by a third isolation (C and D), grown on two different agar media, Molisch's (C) and carrot (D). ×1

Culture 8, in 1-week-old cultures on potato-dextrose agar slants held for a week at constantly maintained temperatures of 0°, 7.5°, 10°, 15°, and 20° C., had produced none or only a little growth at 5°, and slight growth at 7.5°. A mucous covering, like that shown in Figure 5, C, was present on all of the cultures grown at these temperatures, being most abundant in the cultures held at 10° and 15°. Practically the same results were obtained with isolations of a number of other Sphaceloma fungi grown in parallel cultures with culture 8. In the cultures of *S. ampelinum*, causing anthracnose of grape (Vitis), it was produced in a noticeably larger quantity than in any of the others, appearing as a thick, clear, glistening mass. After 12 days at a temperature of 20°, the mucous covering was still present in only this one species. At any one temperature all of the different isolations grew at about the same rate.

ASSOCIATED FUNGI

A Fusarium, identified by C. D. Sherbakoff as *F. fructigenum* Fr., was observed both at Orlando and at Gainesville, Fla., as forming fluffy pinkish borders about the scab lesions or galls, often covering all but the central area occupied by the Sphaceloma. (Fig. 2, C, *a*, and D, *a*.) This may be the Fusarium that J. B. Ellis found on early specimens of scabbed citrus from Ocala, Fla., sent him by Scribner, and which Ellis thought might be the cause of the disease (*17, p. 182*). Two other secondary fungi occasionally seen on scab lesions are *Colletotrichum gloeosporioides* Penz. and a pycnidial fungus which is possibly a species of Phoma. Superficially both resembled dark acervuli of *Sphaceloma fawcettii* such as are shown on the upper

surface of the section illustrated in Figure 3, D. The first secondary fungus grew on the upper side of necrotic leaf lesions and the second on the gall type of lesion on young stems. At Orlando, on scab lesions on an old lemon fruit still hanging on the tree, were coarse conidial fructifications identical with or resembling those usually referred to *Cladosporium herbarum* Massee. It was not determined whether they were identical with a Cladosporium common on the Orlando citrus collection, which sometimes developed in original isolation cultures of *S. fawcettii*.

The Cladosporium referred to previously as occasionally developing in original isolation cultures of *Sphaceloma fawcettii* was readily distinguishable from this organism. Rather delicate as seen in culture, its pale grayish colonies developed much faster than those of *S. fawcettii* and were noticeably less stromatic. The different types of conidia produced by the Cladosporium in culture were sometimes indistinguishable from those of *S. fawcettii* as developed on citrus in the rutaceous collection. The usual absence of conidial fructifications in cultures of *S. fawcettii* has been noted previously (*25*). This similarity included not only size, color, and general form, but also methods of conidial formation, among them the prompt development of hyaline oval or elliptical biguttulate conidia when the elongate colored conidia were transferred from a less humid to a more moist environment. Although generally longer than the Cladosporiumlike conidiophores of *S. fawcettii*, they were of about the same width.

INOCULATIONS

Through inoculation tests the pathogenicity of culture 8 on lemon was proved in 1924 (*9*). In parallel inoculations with other Sphaceloma isolations representing the same or other species, this culture has since been employed in inoculation tests on four other kinds of citrus, namely, rough lemon (*Citrus limonia*, No. 7610[7]), grapefruit, Thomasville citrangequat (*Fortunella marginata* × Willits citrange, No. 48010), and Cuban shaddock (*Citrus* sp. hybrid, No. 11893). The rough lemon was inoculated in 1926, the others in 1927. The inoculations were made in greenhouses at the Arlington Experiment Farm, United States Department of Agriculture, or in those of the department of plant pathology, Cornell University, none of which at the time contained any citrus other than that employed in the tests. Leaves were inoculated in all cases. The method of inoculation was essentially that described by Winston (*25*). Small stromatic masses of the fungus, developed in culture, were placed on bits of wet cotton so applied that the fungus came in contact with the leaf surface, and the whole was then wrapped in waxed paper. After about 48 hours all of the coverings were removed. The checks were treated in the same manner except that no inoculum was used. From two to seven individual inoculations, with as many checks, were made for each kind of plant. On Cuban shaddock, inoculations were also made with culture 71, as representative of the other distinct but less usual type of cultural growth. The final readings on infection were made from 18 to 21 days after making the inoculations.

[7] The serial numbers cited are those of crop physiology and breeding investigations, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, by which the plants to be inoculated were contributed.

With the exception of the citrangequat, infection resulted in all the inoculated plants, although not on every leaf inoculated. No reisolations were made. The checks remained uninfected. Of seven leaves of Cuban shaddock inoculated with culture 71, four became infected; and of seven leaves of the same variety inoculated with culture 8, at least one became infected. In addition to establishing the pathogenicity of these two strains of *Sphaceloma fawcettii* on certain rutaceous hosts, these inoculations serve to show that an isolation grown continuously in pure culture for as many as 11 years is still capable of producing infection. They also furnish a record of the fact that two isolations from different sources, markedly different in their gross cultural characteristics, were not physiologically different in their ability to infect a common citrus host, Cuban shaddock, on which no infection had previously been observed (*26*).

SUMMARY

This paper presents data pertaining to the development of conidia and conidiophores of *Sphaceloma fawcettii*, the causal fungus of citrus scab in Florida, especially as based upon the superficial development of the fungus in Florida on spring and autumn growth of leaves of sour orange, grapefruit, Tahiti lime, and young fruit of grapefruit. Other data included deal with cultural studies, inoculation tests, and secondary fungi associated with this fungus. Mention is made of the occurrence of *S. fawcettii* in Florida as early as 1878 and of probably the same fungus in South America in 1882 as well as its distribution in Java. The inoculation tests proved the pathogenicity of the organism on citrus after it had been grown in culture for a period of 11 years, and also its power to infect Cuban shaddock. In connection with the cultural studies of the fungus, colonies of two markedly different types are reported.

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THE MECHANISM OF SEX IN UROMYCES APPENDICULATUS AND U. VIGNAE[1]

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INTRODUCTION

The statement has been often reiterated that the spermogonia of the Uredineae are functionless male organs, having at most a doubtful physiologic value. Since the work of Blackman (*7*)[3], Blackman and Fraser (*8*), and Christman (*10*, *11*), and later confirmation by Olive (*32*, *33*), Dodge (*20*, *21*), Fromme (*22*, *23*), and others, it has generally been held established that fertilization in the rust fungi consists in a fusion of equal gametes in the aecial primordium which results in the formation of a binucleate basal cell from which the chain of binucleate aeciospores and intercalary cells is produced. The fact that many investigators previous to Blackman and Christman, and a few since, have failed to observe the occurrence of such fusions shows the difficulty with which they may be observed and also their doubtful frequency. Later cultural work by Craigie (*13*, *14*, *15*) indicates that the spermatia must be assigned some part in the fertilization process.

In demonstrating the heterothallism of *Puccinia graminis* Pers. and a few other rust organisms, Craigie has shown that the nectar containing the spermatia is directly associated with maturation of spores in the aecium. He has shown that when nectar from a + spermogonium is applied to one portion of an undeveloped − pustule, the treated section of the pustule will form mature aecia while the untreated area remains sterile. Craigie's results have been confirmed in part by the writer, using the aecial stages of *Uromyces appendiculatus* (Pers.) Fries from beans (*Phaseolus vulgaris* L.) and *Uromyces vignae* Barclay from the cowpea (*Vigna sinensis* (L.) Endl.). The two forms have only recently been distinguished by Fromme (*24*) on the basis of a slight difference in spore characters. They are both full-cycle rusts, showing little if any physiologic specialization.

METHODS

A method of inoculation was used in which the teliospores were germinated on water drops before they were applied to the host leaves. In this way the sporidia were separated and a maximum number of isolated sori obtained. By this method the first rust flecks appear on the third day after inoculation. Small droplets of nectar containing spermatia appear about the sixth day on the upper leaf surface.

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[3] Reference is made by number (italic) to Literature Cited, p. 585.

In case fertilization takes place, aecia follow on the lower surface about the tenth day. No careful attempt was made to eliminate insects entirely, but it was conclusively shown that, where nectar was transferred by hand, the aecia became conspicuously swollen by the third day after transfer, whereas maturation was delayed several days or failed to take place where no such transfer was made. Material for cytological examination was killed and fixed in Carnoy's and in Flemming's weaker solution and embedded in commercial paraffin. The best results were obtained with material cut as thin as it could be conveniently sectioned, 4μ to 5μ—never more than 7μ. In thick sections the maze of cells is entirely confusing and the staining is less satisfactory. The triple combination of safranin, gentian violet, and orange G, prepared according to Colley's methods (*12*), was used throughout for staining. In a few cases erythrosin was used in combination with the triple stain in order to bring out the vegetative nuclei of the fungus.

An examination of sectioned material showed the presence of binucleate cells in the primordium within 24 hours after transfer of spermatia. On the third day after transfer the aecia contained extended chains of aeciospores. Material of the same age, but where nectar had not been transferred, showed no binucleate cells, except in rare instances where insect transmission of spermatia may have taken place.

ARRANGEMENT OF SEXUAL ORGANS

Figure 1, A, shows the usual arrangement of sori in the gametophytic stage of *Uromyces appendiculatus* on bean plants. In bean rust the aecial primordia form in a ring in the mesophyll tissue of the host leaf, and the spermogonia form a cluster of sori opening on the upper leaf surface. The arrangement is similar in the cowpea rust, but the aecial primordia do not form so definite a ring. A few spermogonia frequently occur on the lower leaf surface and a few aecia on the upper. Pustules on leaf veins or petioles usually show a pronounced linear progression.

The first macroscopic evidence of fertilization in the aecia is a swelling of the pustule on the lower surface of the leaf. The spermogonia apparently cease functioning immediately on fertilization of the aecia. This seems to be true whether the fertilization takes place at an early stage or is long delayed. In case of delayed fertilization the spermogonia continue to produce nectar until the host tissue is exhausted. In one such case of delayed fertilization a secondary ring of exudate was found surrounding the primary cluster. This pustule was not examined microscopically, but if there was present a secondary growth of spermogonia it indicates a remarkable adaptiveness for attracting insects and securing fertilization. Sections of material on which nectar had been transferred by hand show a film of spermatia in a gelatinous matrix well distributed over the epidermis at the infected area.

This obvious association of the spermatia with fertilization in the aecium encouraged a detailed cytological study of the process. The presence of hyphae conspicuously projecting through the stomata of the infected area and in close proximity to the spermatia-containing nectar was the first fact in a chain of evidence which has led the writer to conclude that there is present in these species of rust a sexual mechanism very similar to that found in the red algae.

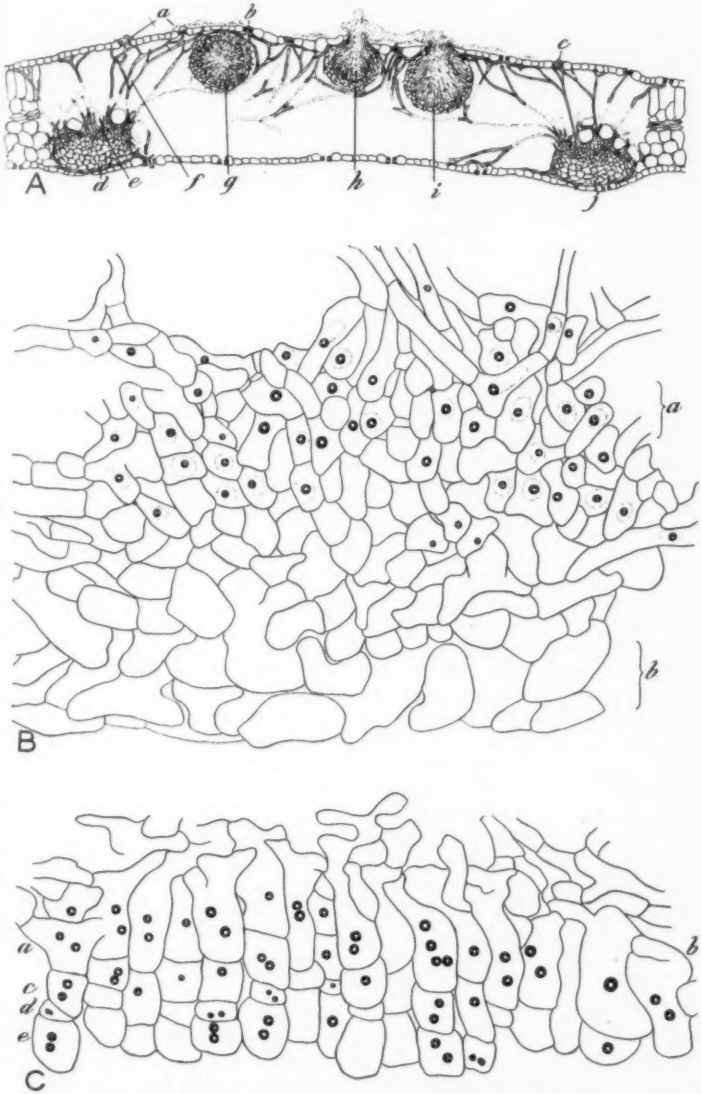

FIGURE 1.—Sexual mechanism in *Uromyces appendiculatus*.[4] A.—Semidiagrammatic drawing of infected leaf area showing relation of male and female elements at the time when fertilization is taking place: *a*, Film of nectar containing spermatia; *b*, tip of gametophytic hypha, ruptured at *c*; *d*, *j*, aecial primordia; *g*–*i*, spermogonia; egg cells at *e*, with branched trichogynes, *f*. × 73. B.—Perplexing arrangement of cells in the aecial primordium, showing egg cells (*a*) and pseudoparenchyma (*b*). × 685. C.—Normal appearance of aecium at an early stage of maturity, showing fertilized egg cells (*a*, *b*), spore initial cell (*c*), intercalary cell (*d*), and young aeciospore (*e*). × 685

[4] In this and the succeeding legends the structures believed to function as egg cells, trichogynes, male nuclei, etc., are thus designated for purposes of brevity and clarity. The terms "fertile cell," "basal cell," and "egg cell" are used interchangeably.

AECIAL PRIMORDIA

A close study was made to determine the structure and orientation of cells in the aecial primordia. These have been described only as tangled masses of hyphae in the early state, with later a differentiation of fertile gametes at the base and sterile buffer cells toward the epidermis of the host leaf. No mention had been found of any definite pattern of growth of these hyphae or of any definite habit of branching until a recent report by Allen (*2*). On casual observation the primordium appears to be no more than as described, but with the use of thin sections (4μ–5μ) definite structures can be traced. These are as shown in Figure 2, B to I. Extended observations have established the fact that this is a general condition in the forms studied. Figure 1, B, shows the baffling appearance of the primordium, but careful focusing reveals within this mass the presence of a larger number of 2-legged uninucleate cells with a constant habit of septation. (Fig. 2, D to G.) Owing to their peculiar orientation (fig. 2, C, G), never more than a few of these branched cells can be seen clearly in the same plane. In the earliest stage in the development of the primordium these cells are set at all angles, but later those nearest the epidermis of the leaf become disorganized and in part compose the pseudoparenchyma. Later, uninucleate cells are formed from the basal cells below and are added to the pseudoparenchyma. (Fig. 3, G, H, K, L, M.) At the point where fertilization would normally occur, the fertile cells become swollen and are crowded together in such a manner as further to obscure the connections with hyphae below. After fertilization, the elongation and continued expansion of the basal cells (fig. 3, I), spore mother cells, and aeciospores force a reorientation, so that the row of basal cells appears in nearly the same plane. (Fig. 1, C.) If fertilization does not take place at the maturation of the first fertile cells, development within the primordium may continue. The result is a chain of cells, each of which in turn could function as a fertile cell. From each cell of the series there extends a lateral, basal stalk, which might be interpreted as a trichogyne, as will be shown later. The terminal cells of the chain are vacuolate and lie within the pseudoparenchyma. Series of three fertile cells occur frequently, but none longer have been observed with certainty. This serial arrangement appears before fertilization, as shown in Figure 3, G, H, L, M, N. A further elaboration in structure may take the form shown in Figure 2, A. Such an extensive proliferation is infrequent in the uninucleate condition.

COPULATORY ORGANS

The hyphae emerging through the epidermis provide an ample mechanism for fusion of spermatia with gametophytic mycelium. It was early discovered that the conspicuous organs in the open stomata (fig. 4, C) found in the first instance are not the most numerous and may not function as trichogynes. They probably represent an overdevelopment after failure to secure fertilization. They are most abundant in old primordia in which fertilization has not taken place, and are entirely absent in primordia fertilized as early as 10 days after inoculation.

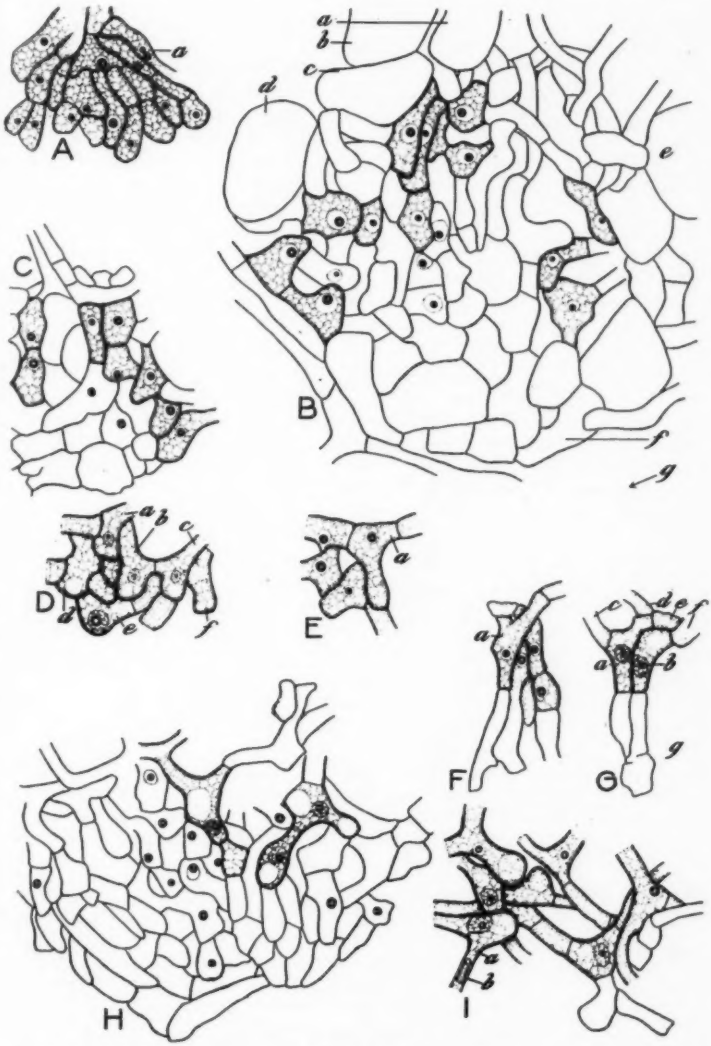

FIGURE 2.—Structure and arrangement of female cells in *Uromyces vignae*. A.—An exceptional case of proliferation before fertilization. Note the binucleate branch (*a*). × 685. B.—Early stage in grouping of egg cells. Those showing the branched structure are shaded. Host cells are shown (*a–e*). Empty cells at *f* will compose the pseudoparenchyma. Lower epidermis is indicated at *g*. × 685. C.—Slightly later stage than B, showing orientation of branched fertile cells (shaded) at lateral edge of a primordium. × 685. D.—Section from base of a primordium, showing structural relation of female organs; fertile cells *a* to *f*. × 685. E.—Group of egg cells showing habit of septation. The longer branch (*a*) usually functions as the trichogyne. × 685. F.—Cells from primordium, showing manner in which the branching of the egg cell (*a*) is often concealed. × 685. G.—Two fertile cells (*a*, *b*) with foot cells (*c*, *d*) and trichogynes (*e*, *f*). Empty cells extending into the sterile tissue at *g*. × 685. H.—A young primordium, showing elaboration in structure of female organs. × 685. I.—Section through lateral edge of a primordium, showing orientation of cells. Trichogyne (*a*) with male nucleus (*b*) just entering the fertile cell. × 685

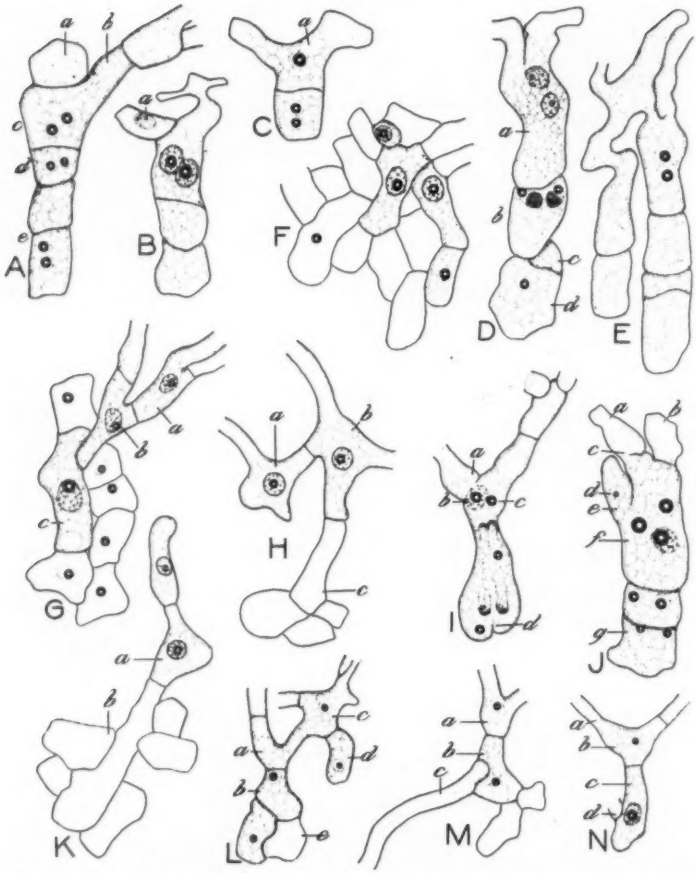

FIGURE 3.—Structure of female cells before and after fertilization in *Uromyces appendiculatus* (A–G, I, J, N) and *U. vignae* (H, K, L, M). A.—Fertilized basal cell (*c*) with stalk cell (*a*) and trichogyne (*b*). Spore initial cell (*d*) with portion of aeciospore chain at *e*. × 830. B.—Similar to A, showing faint, disintegrating nucleus in the branch at *a*. × 830. C.—Recently fertilized egg cell. Area at *a* shows position of second nucleus. Note similarity to F and N below. Stalk cell and trichogyne can not be distinguished. × 830. D.—Same material as C, showing branched basal cell (*a*), spore initial cell (*b*) with paired nuclei preparing to divide, and intercalary cell (*c*), with young aeciospore (*d*). × 830. E.—Linked basal cells after each has begun to produce a chain of spores. Septations have been dissolved. × 830. F.—Typical appearance before fertilization of branched basal cells shown in A, B, C, and D. × 830. G.—Linkage between basal cells before fertilization. After *a* and *b* had each begun to produce a spore chain they would probably appear as in E, above. What is possibly a third fertile cell of the series is shown at *c*. × 830. H.—Another type of linkage between egg cells (*a*, *b*) previous to fertilization, showing empty cells (*c*) extending into the pseudoparenchyma. × 830. I.—Branched egg cell soon after fertilization, showing that *a* is probably not a fusion cell. (Cf. fig. 8, B.) The nuclei, *b*, *c*, were distinct in stain and structure. What is probably the beginning of proliferation appears at *d*. × 830. J.—Same material as I, showing fertilized basal cell (*f*), with trichogyne (*a*), stalk cell (*b*), and lateral fusion cell (*e*) with a small, faint nucleus (*d*). Note the break in the septum at *c*. The adjacent section shows a third nucleus in cell *g*. × 830. K.—Early appearance of egg cell (*a*), showing similarity to Allen's "irregular swelling." Portion of sterile tissue at *b*. Compare with L, M, and N. × 830. L.—Linkage between egg cells (*a*, *c*, and probably *b* and *d*). Sterile cells at *e*. Compare with H. × 685. M.—Linkage between egg cells (*a*, *b*). The trichogyne (*c*) extends into the pseudoparenchyma. Compare with N. × 685. N.—Egg cells (*b*, *c*) with trichogynes (*a*, *d*). This serial growth of fertile cells accompanies delayed fertilization. Compare with C. This indicates also a possible interpretation of J, in which *e* of J would be a second trichogyne, corresponding to *d* of this figure. × 685

But in addition to these conspicuous organs there are other ways in which the gametophytic mycelium may make contact with spermatia distributed on the surface of the host leaf. There are numerous cases in which the spermatia have entered through the open stomate and lie in contact with the mycelium. Figure 5, B, shows such a case. This is situated directly below an aecium that has just begun to produce binucleate cells. Hyphae lying parallel to the epidermis (fig. 5, C, D) are frequently seen to follow the periphery of the primordium or aecium and are lost in the tangle of mycelium at the base of the aecium.

The stomatal inclusions of the type shown in Figure 4, A, are still more numerous. These have no special structure but are often much constricted in passing between the guard cells and are swollen at the extremity. In material sectioned previous to fertilization these hyphal tips take a deep violet stain. In sections of material containing fertilized aecia the tips are frequently found shriveled and disorganized, showing only the orange of the triple stain. (Fig. 4, I, K.) In aged, sterile material—that is, material of unfertilized aecia three weeks or more after inoculation—there are found tips of hyphae projecting out between the guard cells, which are deeply stained with safranin. (Fig. 5, E.) This staining reaction is characteristic of disintegrating tissues or organs which have passed the functioning stage and become senescent (*4*).

In certain material numerous hyphae extrude directly between the epidermal cells. (Fig. 6, A to F.) As soon as these structures were found much of the earlier material was reexamined and many such cases discovered, all of which had previously been overlooked. They are either absent or not at all apparent in some material. They are most general on host material where the constrictions between epidermal cells are especially pronounced. In such material they may be so numerous as to provide alone a sufficient mechanism for copulation.

These facts concerning the occurrence of gametophytic hyphae protruding through the epidermis of the host leaf are by themselves not particularly suggestive. Such a thing might be expected of any internal parasitic fungus. But it is important to mention that such erumpent hyphae are not found in the telial stage of bean rust. The possibility was borne in mind that these isolated hyphae projecting beyond the epidermis might be nothing more than paraphyses or sporophores of undeveloped or reduced spermogonia. As a matter of fact what have been interpreted as much reduced spermogonia do occur rarely. One of these is represented in Figure 5, A. Except for a considerable enlargement, these appear to be structurally identical with spermatiophores present in a mature spermogonium. They are certainly homologous. From their staining reaction and nuclear condition it is possible that they do not have the same function as the hyphae described above. It should be emphasized, however, at this point that all these structures are borne on the same thallus, and the habit of septation and branching, as shown in Figure 6, H, is constant throughout all organs of the parasite. All such special structures may be said to arise from separate internodes of the same thallus and are in a sense homologous.

Other facts concerning the time and condition of the appearance of these stomatal hyphae and their association with other parts of

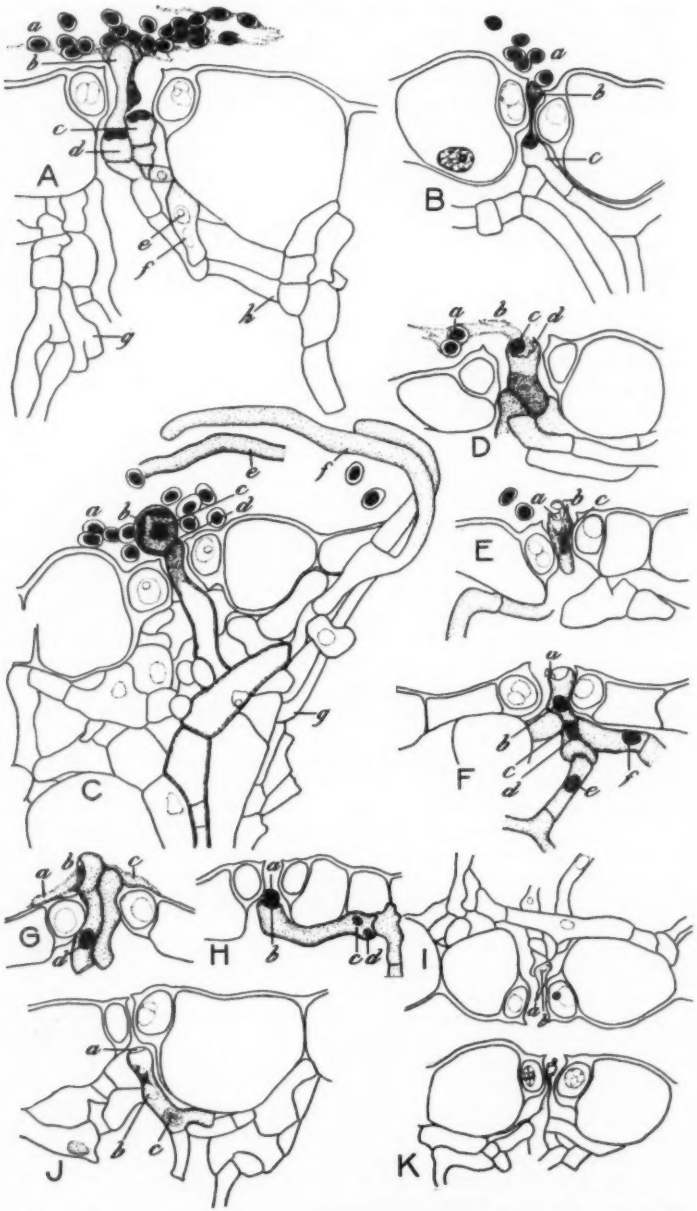

FIGURE 4.—Copulatory organs of *Uromyces appendiculatus* (A–E, H–K) and *U. vignae* (F, G)

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the sexual mechanism, added to the mere fact of their occurrence, indicate that these hyphae are the tips of functioning trichogynes. At an early stage previous to fertilization, when the aecial primordia are in process of formation, that is, 8 to 12 days after inoculation, no hyphae are found in the stomata or protruding between epidermal cells. Instead, numerous elongated hyphal branches are found with their growing tips directed toward the epidermis and often just entering the substomatal cavity or deflected in the constrictions between epidermal cells. (Fig. 1, A.) The appearance of these strands suggests that they are growing toward the region on the epidermis where spermatia are distributed. In this early stage the primordium is a somewhat loosely formed structure. (Fig. 2, B.) At this time outgrowths from the fertile cells which could be interpreted as developing trichogynes are frequently found. (Fig. 2, H, and fig. 3, K, L.) Nectar transfer at this stage results in the fertilization of a few basal cells in some, but not all, of the primordia. The numerous chains of aeciospores which result arise from a proliferation of the few fertilized basal cells. (Fig. 7, D.) In such material cell fusions of various types are common. In exceptional cases there appears to be a rather general dissolution of cell walls. These fusion may or may not be accompanied by migration of nuclei. Many of these fused cells have the identical appearance of fusion cells described by other observers (*10*, *22*) and interpreted by them to be "sex fusions."

At a later stage the hyphal tips are found projecting between the guard cells of open stomata or between epidermal cells. They are always conspicuously more numerous in the vicinity of spermogonia, but appear on both the upper and lower epidermis. No attempt has been made to determine the possible effect of humidity and light on the development of erumpent hyphae. It is possible that under conditions where stomata would normally be wide open the spermatia would enter freely so that any considerable extrusion of a copulatory organ would be unnecessary. (Fig. 5, C.) As a matter of fact in one lot of material, where the plants were kept in moist chambers for 48 hours following transfer of nectar, the sectioned material showed an almost entire absence of hyphae protruding beyond the plane of the guard cells. Spermatia, however, were frequently found within the

EXPLANATORY LEGEND FOR FIGURE 4

A.—Partially sterile infection 36 days after inoculation. Stomatal hyphae (*b–d*) associated with mass of nectar and spermatia (*a*). The hypha (*b*) appears to have a gelatinous sheath. Note the deeply stained receptive spots and the swollen tips of *c* and *d*. Faint nuclei (*e*, *f*) and gametophytic hyphae (*g*, *h*). × 830. B.—A possible germinated spermatium (*b*), but more probably a prolongation of the terminal cell of the trichogyne (*c*). Spermatia at *a*. × 830. C.—Abnormal development of copulatory organ: *a*, Spermatia; *b*, gelatinous sheath. The nuclei at *c* and septation at *d* are both uncertain. Paraphyses (*f*) projecting from ostiole of spermogonium (*g*), and section of broken paraphysis (*e*). × 830. D.—Terminal cell of trichogyne, showing ruptured tip (*d*) with the male nucleus (*c*) just entering. In size and staining reaction *c* is identical with the nuclear contents of spermatia at *a*. Nectar at *b*. Compare with F and G. × 830. E.—Severed tip of trichogyne (*a*), with male nucleus present as an indefinite mass at *c*. The discarded shell of the spermatium is seen on the lip of the ruptured hypha (*b*). Compare with F. × 830. F.—Tip of trichogyne with portion of spore case caught on the lip of the ruptured hypha (*a*). The male nucleus (*b*) is scarcely distinct from cell nuclei (*d–f*). The septation at *c* was not clear. × 685. G.—Terminal cells of two gametophytic hyphae. One hypha ruptured at *b*. Nectar is seen at *c*. The exudate (*a*) may be partly composed of cytoplasm from the ruptured hypha. The male nucleus may be present at *d*. × 685. H.—Terminal cell of a gametophytic hypha with a spermatium resting in a slight depression at the tip (*a*). The receptive spot (*b*) is stained violet. Two indistinct bodies (*c*, *d*) may be nuclei. × 685. I.—Shriveled tip of disintegrating trichogyne (*a*) from material with mature aecia. Nuclear material at *b* is probably remnant of cell nucleus. × 685. J.—Substomatal hypha with ruptured tip (*a*). Two indefinite nuclei are present (*b*, *c*). The former is probably the male nucleus and the latter the usually indistinct cell nucleus. Compare with Figure 6, G. × 685. K.—Ruptured and shriveled tip of a trichogyne from material with mature aecia. Such disintegration normally follows passage of the male nucleus. Compare with J. × 685

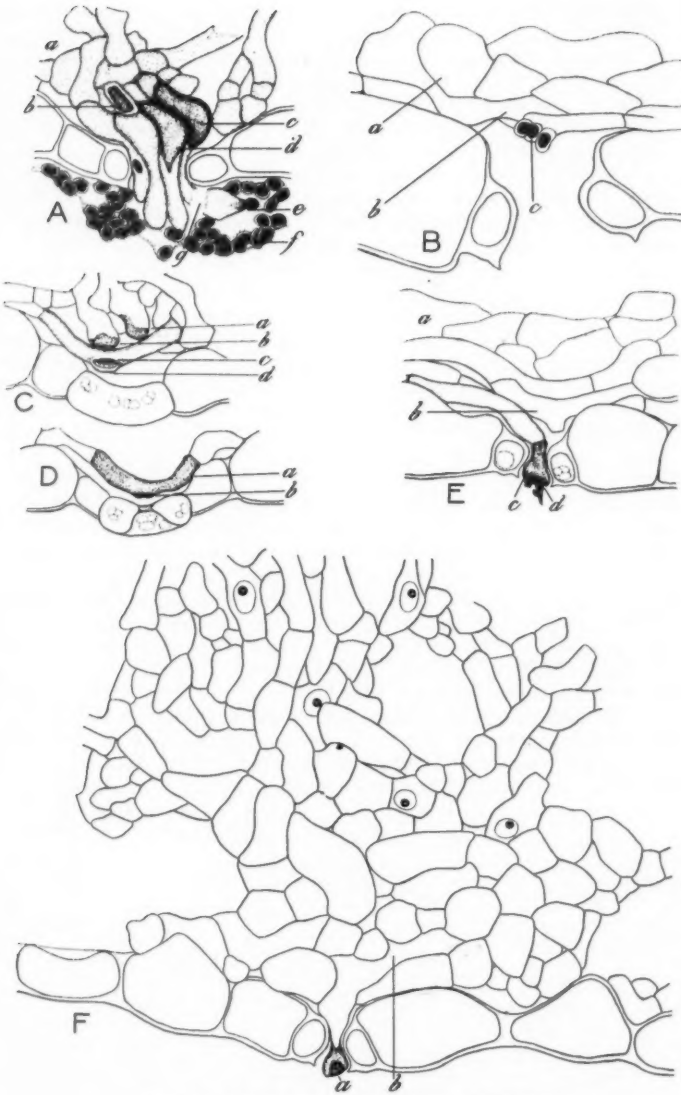

FIGURE 5.—Stomatal structures of *Uromyces appendiculatus*

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open stomata and in contact with the hyphal tips, occasionally appearing to be in process of fusion with the hyphae. On the other hand, material from cowpea leaves cultured in Petri dishes showed high development of erumpent hyphae. The most elaborate development of these organs is found in unfertilized material killed 17 days or more after inoculation. As many as three have been seen protruding through the same stoma. In a number of cases erumpent hyphae have been found growing along the surface of the leaf for some distance. (Fig. 6, C.) The hyphal tips normally take a deep stain, usually violet in the earlier stages and safranin in the later stages. Where the whole terminal cell is not stained heavily, there is in almost every case a darkly stained area at the extremity which at times has the appearance of nuclear material, but more probably represents a receptive spot, possibly such as is found on the unfertilized female cells of other organisms, for example, Vaucheria, and the eggs of various vertebrates.

A study of the internal mycelium reveals a peculiar method of branching by which means the gametophytic hypha progresses along the host epidermis and emerges at any convenient outlet which might be by chance in the region of spermatia. (Fig. 1, A.) Staining reactions indicate that as later branches are formed the earlier ones become vacuolate. The strands usually incline upward and toward the spermogonia opening on the surface of the leaf at the center of the pustule. They may, however, form a parallel weft of mycelium beneath the epidermis, extending for a considerable distance away from the center of the infected area. These subepidermal strands may become very numerous where fertilization has not taken place promptly. The strands are highly septate and the cells uninucleate. The internodes are of irregular length and at times quite prolonged. (Fig. 6, H.) In certain material the presence of a second nucleus is distinguished in individual cells. (Fig. 6, I.) The nuclei stain very poorly and are usually seen with difficulty. They are often considerably elongated and in some cases appear to be partially disorganized. Some possess small nucleoli, others do not. After maturation of the aecia, most of the surrounding hyphae assume an empty and lifeless appearance. In old sterile material the hyphae undergo numerous and peculiar forms of branching and anastomosing.

DISCUSSION OF SEXUAL MECHANISM

Assuming that fertilization does take place by means of a trichogenous hypha extending from the base of an egg cell and terminating at the surface of the host leaf, one must conclude, first, that fusions of cells of parallel hyphae, with accompanying migration of nuclei, when they do occur, can not constitute fertilization. In view of the

EXPLANATORY LEGEND FOR FIGURE 5

A.—Group of hyphae representing what may be a much reduced spermogonium: *a*, Gametophytic mycelium; *b-d*, deeply stained areas; *f*, mass of nectar and spermatia. A possible fusion of hypha tip with a spermatium is at *e*, and a break in the hypha at *g*. × 830. B.—Open stoma beneath an aecium, showing spermatia (*c*) lying in contact with mycelium (*b*) at border of pseudoparenchyma (*a*). Compare with F. × 830. C.—Gametophytic hyphae underlying a stoma, showing a rupture at *c*. The regions *a*, *b*, and *d* are brightly stained with orange G. × 685. D.—Similar to C. The cell *a* is stained dark violet, while the region at *b* is still more deeply stained. × 685. E.—Old and partially sterile infection with pseudoparenchyma shown at *a* and mycelium at *b*. The lifeless terminal cell (*c*) has taken a deep safranin stain. The formless material (*d*) may be part of the ruptured hypha tip. × 830. F.—An aecial primordium showing a hypha projecting through an open stoma with a possible fusion with a spermatium at *a*. The cell *b* is apparently a part of the pseudoparenchyma. Branched egg cells are shown with nuclei. × 830

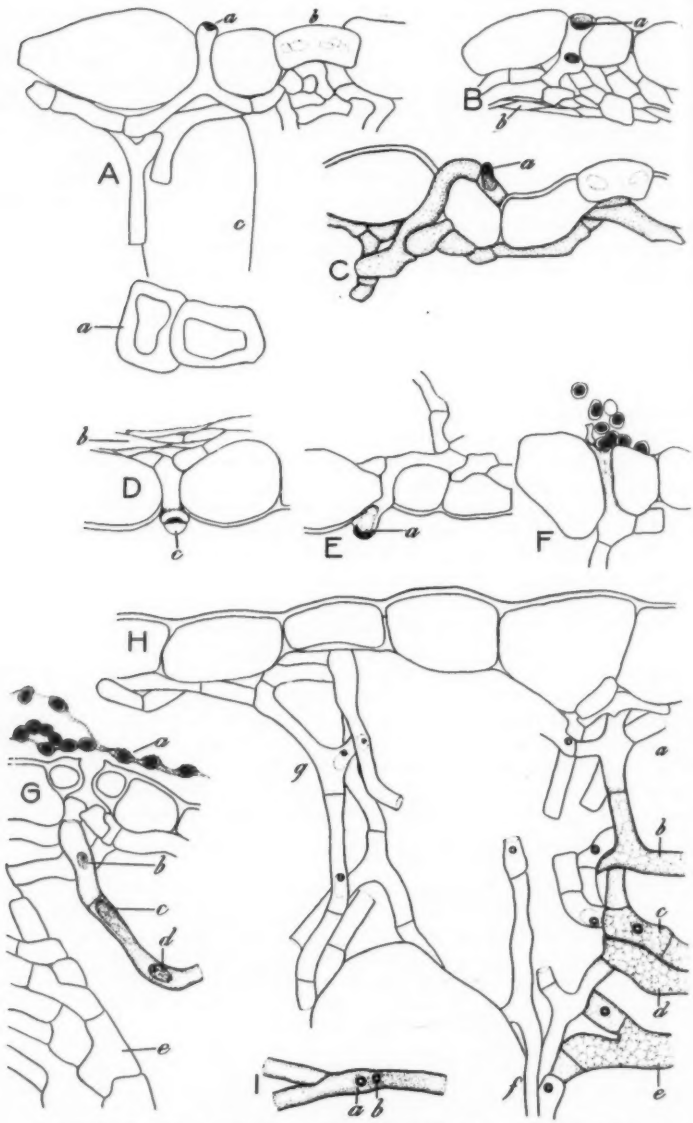

FIGURE 6.—Copulatory mechanism in *Uromyces appendiculatus*. (F is from *U. vignae*)

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many published accounts there can be no question that parallel cell fusions do occur in a considerable number of rust forms. However, the writer has recently examined slides of *Kunkelia nitens* (Schw.) Arth. and is prepared to question many of the conclusions drawn from the observations made on this and similar forms. While structures resembling fusion cells are very numerous on certain of these slides, in many cases at least the manner of junction of the cells is such that they can not, from the standpoint of morphology alone, be interpreted as fusion cells. The evidence indicates that cell fusions occur in the two forms included in the present study, but occur with frequency only when conditions are unfavorable for best development and nonconducive to fertilization in the parasite. The few clear cases of a nucleus in actual process of migration between cells of parallel hyphae show a nucleus migrating into a cell which is already binucleate. (Fig. 8, A.) Parallel cell fusions passed unnoted by investigators previous to the work of Blackman and Christman; since then opinions have been divided as to their actual occurrence and significance. Yet for the most part Christman's conclusions have been accepted. The extreme height of conviction is amply demonstrated in the report by Fromme on sexual fusions in the flax rust, *Melampsora lini* (Pers.) Desmaz. (*22*). On the other hand, Allen (*2*) and Kursanov (*29*) state definitely that they find no indication of fusion in their material of *Puccinia graminis*, and so make advisable a complete reexamination of rust forms in which cell fusions are reported as constituting fertilization.

In the second place, it must be concluded, on the above assumption, that the row of binucleate basal cells present in the mature aecium, and stated to be 2-legged fusion cells by previous observers, are not necessarily fusion cells but are structures which may be present in the primordium previous to fertilization, that is, in the uninucleate condition. This is clearly the case, as a comparison of Figure 3, A to E, with Figure 3, F, G, and Figure 8, B, will show. It is readily seen that uninucleate branched cells present in the primordium might easily be interpreted as 2-legged fusion cells when in the binucleate condition. It is true that these basal cells form vegetative fusions with parallel hyphae in particular cases. In Figure 3, J, the two branches of the original uninucleate basal cell are shown in addition to the lateral fusion cell. However, the possibility should not be overlooked that this lateral cell (*d*) may represent a second trichogenous hypha. Perhaps normally a cross wall would have divided

EXPLANATORY LEGEND FOR FIGURE 6

A.—Hypha projecting between epidermal cells, showing deeply stained receptive spot (*a*); stoma at *b*, and host palisade cell at *c*. × 685. B.—Hypha projecting between epidermal cells beneath a mature aecium, showing ruptured tip (*a*), and pseudoparenchyma (*b*). Note presence of nucleus in the terminal cell, as contrasted with A. × 685. C.—Gametophytic hypha growing on surface of host leaf. The nucleus (*a*) may represent a spermatium in process of fusion with the hypha. × 685. D.—Hypha projecting between epidermal cells beneath a mature aecium, showing peridial cells (*a*) with crushed cells of the pseudoparenchyma (*b*). The empty violet-stained structure (*c*) is probably not a spermatium. × 685. E.—Probable fusion of a male nucleus with tip of trichogyne protruding between epidermal cells (*a*). Compare with C. × 685. F.—Ruptured tip of hypha of *U. vignae* appressed between epidermal cells, showing close association of spermatia. × 685. G.—Gametophytic hyphae at a stoma near ostiole of a spermogonium, showing migrating male nucleus in trichogyne (*c*), and cell nuclei, disintegrating (*b*) and still functional (*d*). The terminal cell of this hypha has probably been sectioned away. Nectar with spermatia is shown at *a*, and border of spermogonium at *e*. × 685. H.—Section from lateral edge of an aecial primordium showing branching habit of hyphae. Fertile cells (*b-e*) are seen at the edge of the primordium, with branches extending out to the epidermis (*a, f*); indicating also the aggregation of hyphae beneath stomata (*g*). × 685. I.—Section of a hypha from beneath an aecium where fertilization is in process. The two nuclei (*a, b*), represented here only by the nucleoli, are distinguished by their staining reaction. The violet-stained nucleus (*b*) is probably the male nucleus. × 685

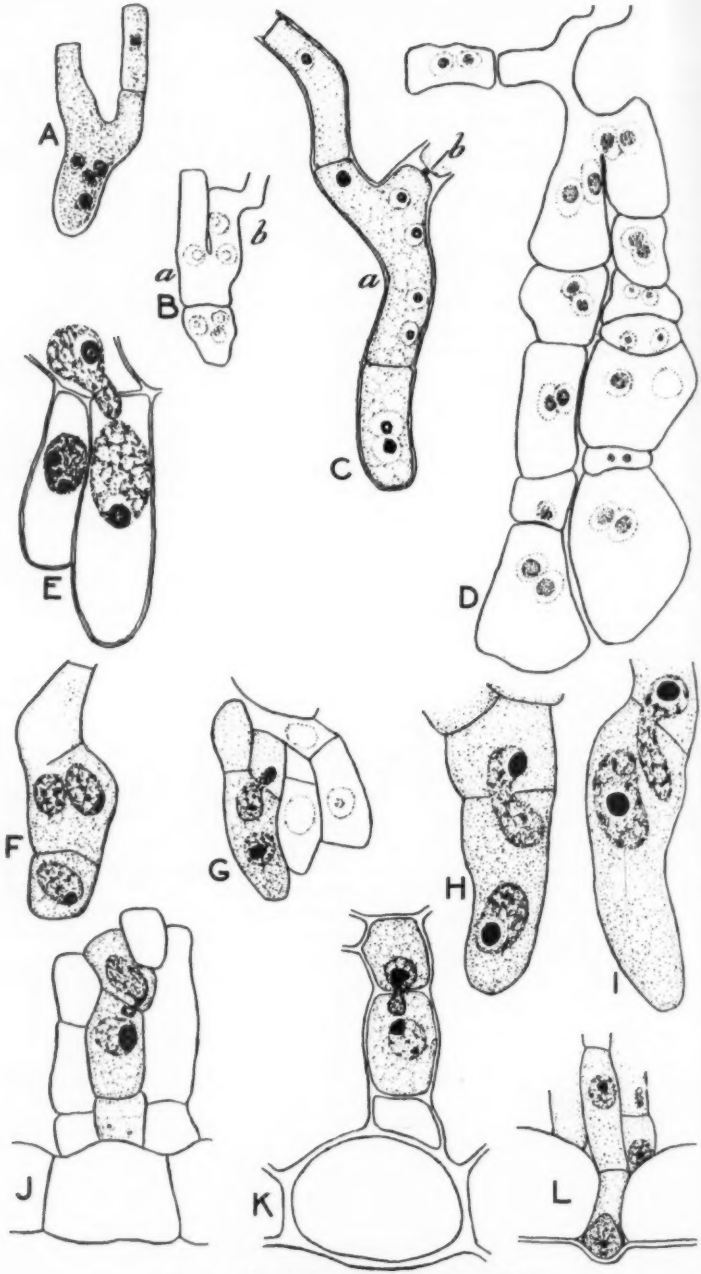

FIGURE 7.—Gametophytic structures in other Uredineae
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the cell (*f*), as has occurred in Figure 3, N. This is drawn from material 17 days after inoculation in which no nectar was transferred. Only a few aecia show binucleate cells. The presence of three nuclei in the basal cell should be noted, as well as the faint nucleus in the lateral cell (*d*). The break in the septum of one foot cell is also suggestive.

In the third place, there should be present, in the primordium, egg cells, each with a branch representing the trichogyne and probably a second stalk representing the foot cell. These structures are present, as described above. After fertilization and subsequent expansion and reorientation, they are represented in the two forms studied by the row of 2-legged basal cells in the aecium (fig. 1, C), similar to what has heretofore been commonly figured.

With a carpogonic structure present such as suggested above, the binucleate condition should be found to arise from the migration of a nucleus from a cell below and continuous with the egg cell rather than from an adjacent cell. While migrations of this sort are not easily seen, they are quite numerous and more regular in occurrence than are migrations between cells of parallel hyphae. Several factors may account for the extreme difficulty in finding the male nucleus in actual process of entrance into the egg cell. For one thing, fertilization of the large number of egg cells in the same aecium, while normally occurring in close succession, does not take place simultaneously. It is extremely unlikely, therefore, that more than a very few male nuclei would be seen in a significant stage of migration in the same aecium. Again it may be that the wall dividing the egg cell from the trichogenous hypha is not formed in some cases until after entrance of the male nucleus, in which case the break in the septum often found following nuclear migration would be absent. (Fig. 7, F, and fig. 8, F.) Migrations of the type described by Blackman (*7*) took place through a very small pore in the septum which was not visible either before or after passage of the nucleus. (Fig. 7, J, K.) Bachmann (*4*) reports a transitory opening in the cross walls of the trichogyne of Collema, while Harper (*26*) reports a similar transitory opening between the oogonium and the conjugation tube in Pyronema. Furthermore, the difficulty of tracing the continuation of egg cell and trichogyne in the compact aecium would prevent finding the whole mechanism in the proper plane except in rare instances.

EXPLANATORY LEGEND FOR FIGURE 7

[These illustrations have been redrawn and inverted]

A.—*Puccinia caricis*. A supposed fusion cell. Compare with Figure 3, N. × 750. (After Kursanov, *29*.) B.—*Cronartium ribicola*. Diagram of an irregular basal cell. A lateral fusion may have taken place at *a*, or the three nuclei may have resulted from the dissolution of a wall at *b*. Compare with Figure 8, C and D. × 850. (After Colley, *12*.) C.—*Uromyces scutellatus*. Hyphal connections of basal cell (*a*) after fertilization. A supposed fusion cell, but compare with Figure 9, G to K. The point *b* may represent a pore in the septum. × 1,500. (After Kursanov, *29*.) D.—*Endophyllum sempervivi*. Two spore chains arising from proliferation of a single basal cell. Magnification not given. (After Hoffmann, *27*.) E.—*Triphragmium ulmariae*. Nucleus migrating into the fertile cell from cell below. × 1,500. (After Kursanov, *29*.) F.—*Phragmidium violaceum*. A binucleate cell, showing the broken septum and the empty appearance of the cell from which the second nucleus has migrated. Compare with Figure 8, F. × 1,300. (After Welsford, *39*.) G.—*P. violaceum*. Migration of nucleus from one of the two basal branches of the fertile cell. Compare with Figure 8, G, and Figure 9, F. × 1,300. (After Welsford, *39*.) H, I.—*Triphragmium ulmariae*. Nuclear migration between continuous cells. × 2,350. (After Lindfors, *30*.) J.—*Phragmidium violaceum*. Early stage of nuclear migration. × 1,350. (After Blackman, 7.) K.—*P. violaceum*. Nuclear migration between cells of same hypha. × 1,350. (After Blackman, 7.) L.—*P. violaceum*. Sterile cell of aecial primordium grown up between epidermal cells and covered only by the host cuticle. × 1,350. (After Blackman, 7.)

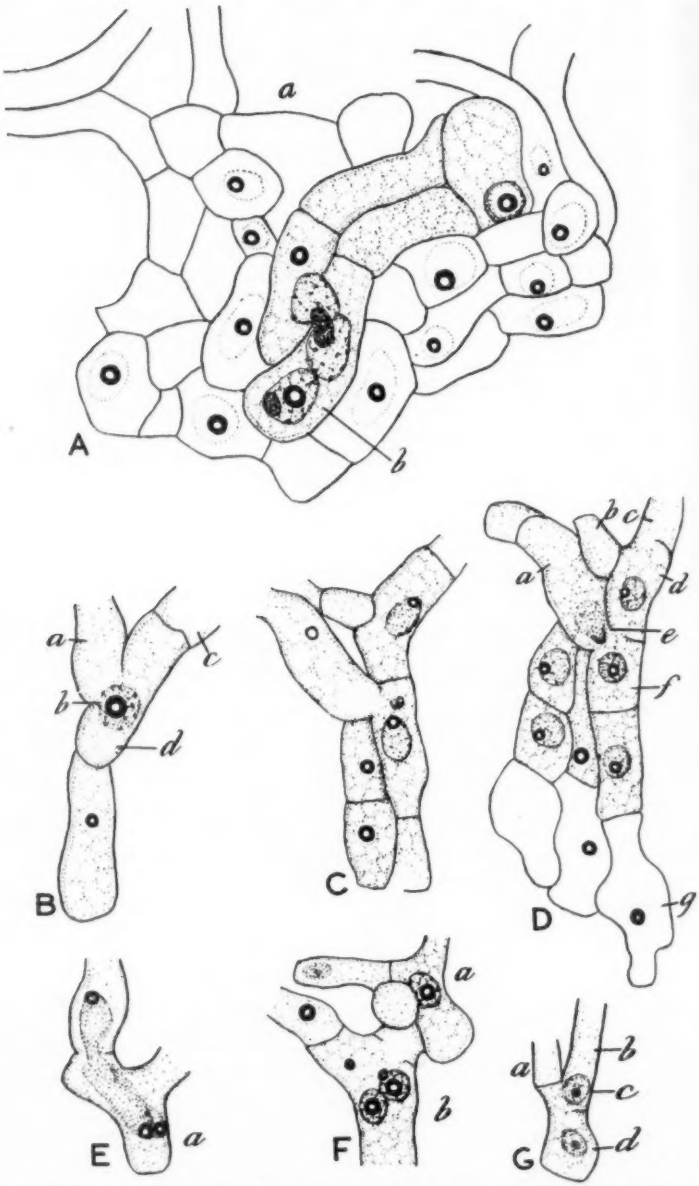

FIGURE 8.—Nuclear migration and fertilization in *Uromyces appendiculatus* (A, B, E, and G) and *U. vignae* (C, D, and F)

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Examples of nuclear migration between continuous cells in bean and cowpea rust fungi are shown in Figure 8, C to G, and Figure 9, A to K. A suggestive fact is the frequent dissimilarity in size and structure of the paired nuclei before the first conjugate division. One is the large, well-defined nucleus with a very large nucleolus found in each of the fertile cells before fertilization; the other is small and poorly defined, often lacking a nucleolus but appearing rich in chromatin material. It should be recalled that the nucleus of the spermatium, while large as compared with the size of the spermatium as noted elsewhere, is a small deeply staining body without a nucleolus, while the vegetative nuclei below the fertile cells, although small, have a large nucleolus and stain no differently from the single nucleus in the fertile cells. Illustrations reproduced in Figure 7 show that migrations of nuclei into the fertile cell from a cell below and continuous with the fertile cell have not passed unobserved in other rust forms.

It would of course be desirable to trace the trichogenous branch extending from the egg cell through the host tissues and mass of vegetative mycelium of the fungus until it terminates in association with male cells. The difficulty of doing this by the usual serial-section method is obvious. In no case has it been possible to follow a particular hypha from an egg cell to its terminus at the surface of the host leaf. The most that has been accomplished is to trace hyphae from the egg cells to a point free from the aecium and to trace hyphae from their extremity in the stomata to a point where they are lost among the cells of the aecium.[5]

Finally, if copulation of spermatia with trichogynes takes place, these should be found in actual process of fusion. In fact many cases have been seen which strongly suggest that a fusion of spermatia with stomatal hyphae is taking place or has taken place. Figure 4 shows various stages in the fusion process. Hyphae showing ruptured tips are especially numerous. (Fig. 4, D, E, F, G, J.) Protected as they are between the host cells, there is no reason to suppose that these ruptures are entirely the result of accident. Numerous also in certain material are the shriveled tips represented in Figure 4, I and K. Spermatia are in close association with the unruptured tip in Figure 4, A. A spermatium in a depression at the tip of the hypha is shown in Figure 4, H. Figure 4, E and F, show what appears to be the discarded spore case of a spermatium caught on the lip of the ruptured hypha. Within the one hypha is found an irregular mass resembling nuclear material, while in the other a definite nucleus

[5] The interpretative nature of structures here designated as "egg cells" and "trichogynes" should be emphasized at this point. Some observers will probably maintain that these egg cells bear no more than a functional relationship to egg cells in algae or other organisms, and that the trichogenous branch is in fact no more than a simple vegetative hypha.

EXPLANATORY LEGEND FOR FIGURE 8

A.—Nuclear migration between cells of parallel hyphae. The cell *b* already contains two nuclei, suggesting a previous migration from another source. The base of the primordium is at *a*. × 1,660. B.—Fertile cell (*d*) previous to migration of male nucleus, showing trichogyne (*a*) and stalk cell (*c*). Such a constriction (*b*) at the junction of egg and trichogyne is frequently found both before and after fertilization. Compare with C and D and with Figure 3, I. × 1,660. C, D.—The same structures as they appear in adjacent sections. Two egg cells (*d*, *f*) in series, with trichogynes (*a*, *c*), stalk cell (*b*), and migrating male nucleus (*e*). A second nucleus in cell *d* is shown in C. Pseudoparenchyma cells are seen at *g*. Compare arrangement of fertile cells with that in Figure 9, E. × 1,370. E.—Nuclear migration between continuous cells. Apparently two nuclei are already present in the egg cell (*a*). × 1,370. F.—Two egg cells, before (*a*) and soon after fertilization (*b*). × 1,370. G.—Male nucleus (*c*) migrating from the trichogyne (*b*) into the female cell (*d*). Stalk cell at *a*. Compare with Figure 7, F and G. × 1,370

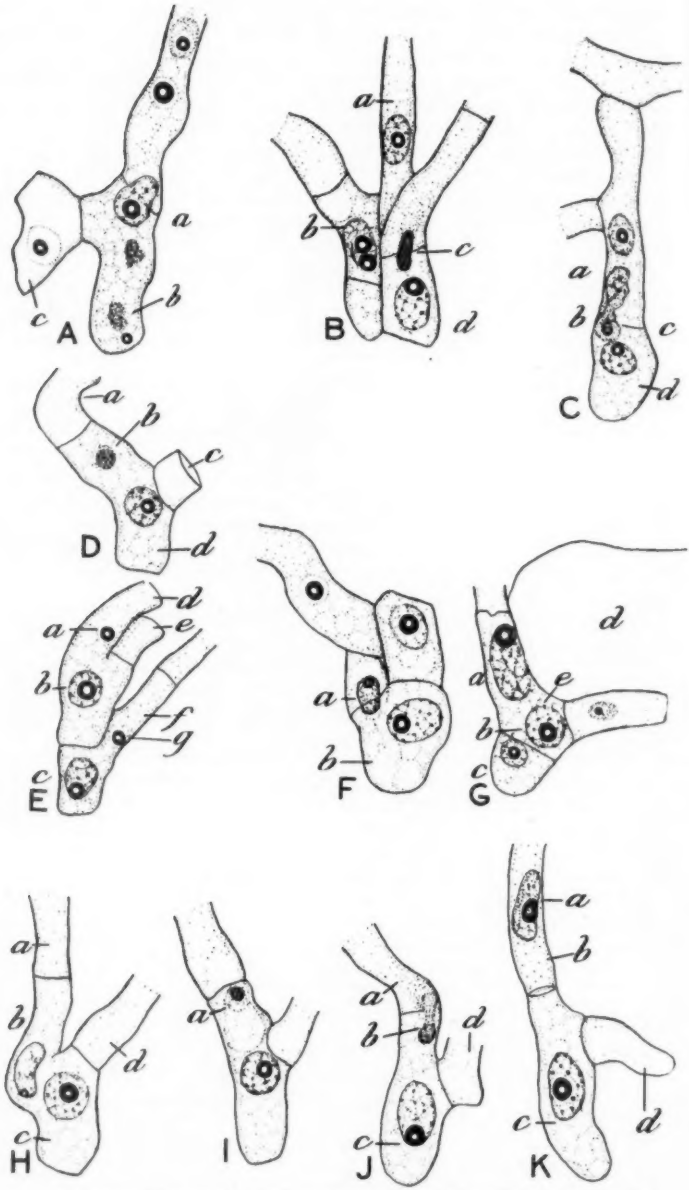

FIGURE 9.—Fertilization in *Uromyces vignae* (A, C) and *U. appendiculatus* (B, D–K)

For explanatory legend see opposite page

is visible, with a second very lightly stained body present. In few cases do the terminal cells of unruptured stomatal hyphae show a definite nucleus, while the cells below commonly have a single poorly stained but usually definite nucleus. The terminal cells of the ruptured hyphae occasionally show two nuclei, usually not well defined. Figure 4, J, shows such a case. The frequent occurrence of two nuclei of different appearance in the same hyphal cell strongly suggests the migration of a foreign nucleus. (Fig. 6, G.) In fact, nuclei have been seen passing through the septa between hyphal cells. A small pore can be seen in many of these cross walls, and in addition they are often found ruptured, supposedly after passage of the male nucleus.

RECAPITULATION AND REVIEW OF LITERATURE

In reviewing the literature on cytology and sexuality in the Uredineae, many of the above observations are found supported by the work of previous investigators. It is believed that the present interpretation will harmonize many conflicting opinions, and that many cases of exceptional behavior in the rusts will come to have a meaning. Several observers have suggested the relationship of the Uredineae to the Rhodophyceae. The uninucleate sterile cells budded off previous to fertilization were regarded as vestiges of once functional trichogynes. It is possible that it has not occurred to anyone to seek for vestiges of a trichogyne at the base of the fertile cell. No one has maintained that the fertile cell in the rust aecium is in a true sense a functional egg cell. Migrations of nuclei into the fertile cells from cells lying beneath and continuous with the fertile cells have been described for a number of species of rust. (Fig. 7.) But in most cases those reporting the occurrence of such migrations have maintained that migrations also take place between cells of parallel hyphae and that either type may constitute fertilization. Apparently, to most of them fertilization consists in a partial or complete fusion of equal gametes. Fromme (*22*) has even described the fusion of as many as four parallel cells to form a 4-legged basal cell. It would seem a point for controversy, then, not only when fertilization begins but when it ends.

The protrusion of gametophytic hyphae through host stomata is associated with at least several other rust forms, as revealed by a careful search of older publications. Recent observers have made scarcely any note of their occurrence, but in their drawings some have come very close to representing such structures. De Bary (*5*)

EXPLANATORY LEGEND FOR FIGURE 9

A.—Male nucleus (*a*) entering the fertile cell while the female nucleus is in process of division (*b*). Note similarity of *c* to *b* of Figure 2, D. × 1,370. B.—Two egg cells, *b* fertilized, and *d* with male nucleus just entering at *c*. The hypha *a* is probably a second branch of *d*. × 1,370. C.—Nuclear migration between continuous cells. Here a wall (*c*) has formed above the stalk cell (*a*). A male nucleus (*b*) is entering the egg cell (*d*). Compare with Figure 7, H. × 1,370. D.—Basal cell (*d*) soon after fertilization, showing trichogyne (*a*), stalk cell (*c*), and male nucleus (*b*). × 1,370. E.—Two egg cells in series and in same stage of fertilization, with male nuclei (*a*, *g*), female nuclei (*b*, *c*), trichogynes (*d*, *f*), and stalk cell (*e*). Compare with Figure 8, C. × 1,370. F.—Male nucleus (*a*) entering female cell (*b*). × 1,370. G.—A fertilized cell (*e*). Under some conditions the male nucleus (*a*) may continue through the wall at *b* so that the cell *c* would function as the basal cell of the spore chain. Host cell is shown at *d*. Compare with C. × 1,370. H.—Basal cell (*c*) soon after entrance of male nucleus (*b*), showing trichogyne (*a*) and stalk cell (*d*). × 1,370. I.—Basal cell just after fertilization, with male nucleus poorly outlined at *a*. × 1,370. J.—Basal cell (*c*) with male nucleus entering at *b*, showing trichogyne (*a*), and stalk cell (*d*). × 1,370. K.—Basal cell (*c*) just before entrance of the male nucleus (*a*), showing trichogyne (*b*) and stalk cell (*d*). H, I, J, and K show remarkably similar structures at different stages of fertilization. × 1,370

in 1887 reported the frequent occurrence of stomatal hyphae. Richards (*35, p. 257*), describing the aecial stage of *Uromyces caladii* on Peltandra, says: "Occasional hyphae were seen protruding out of the stomata, but they did not connect with any of the primordia, and showed no evidence of any specialization." Klebahn (*28*) reports seeing wefts of hyphae in open stomata from several species of Aecidium. He finds them often swollen at the tip and frequently associated with spermatia, but he concludes that they have no sexual function. He even speaks of artificially spreading spermatia over the leaf area where aecial primordia were awaiting fertilization, and concludes that they do not influence the maturation of the aecia. No reference has been found to this phase of Klebahn's work in any publication. Kursanov (*29*), Blackman (*7*), and a few others have shown gametophytic hyphae associated closely with an aecium crowding apart the epidermal cells. Allen (*2*) shows one hypha which has protruded between epidermal cells and extended for some distance along the surface of the host leaf. Her interpretation of the structure indicates that it has no such function as a trichogyne. There might be mentioned parenthetically a situation found in *Polystigma rubrum* DC. The sexual condition in this ascomycete is of a doubtful nature similar to that in the rusts, and Blackman and Welsford (*9*) describe gametophytic hyphae projecting through the epidermis at a very suggestive stage. In this connection it is well to add that Moreau and Moreau, in a recent paper (*31*), deny the occurrence of a true fertilization in any of the Ascomycetes or lichens. According to these investigators, copulation by means of a trichogyne in any of these forms is a myth.

Blackman perhaps more than any other has appreciated the fact that the spermatia of the rusts are male cells (*7*). He regards them as now functionless. It appears from his statements that all that is lacking to make them functional is a mechanism for bringing the spermatia in contact with the female cells at the base of the aecium. He says: "That the spermatia, though perfectly functionless, should still be produced by the majority of forms and in such great abundance is certainly a very striking phenomenon." He also notes the similarity of rust spermogonia to those of Collema, in which the sexual nature of the spermatia has been shown by Stahl (*37*) and Bachmann (*3*). More recently Dodge also has emphasized the sexual nature of the spermatia of the Uredineae (*19, p. 1055*). He states: "There being no organ such as an egg apparatus in the rusts, the spermogonia, though they are so well developed in many species, can not carry out their primary male sexual function." That the rust spermatia are not perfectly functionless has been proved by confirmation of Craigie's work. The presence of structures functioning as an egg apparatus now appears probable.

As Blackman states, the structure of the spermatia alone indicates their nature as male cells. The fact that they may produce germ tubes does not necessarily disprove their sexual nature. According to Blackman (*7*) a similar vegetative potentiality occurs in the gametes of some algae. Allen (*2*) recently reports what she interprets as germinating spermatia of *Puccinia graminis* on the leaves of barberry. She even suggests that the germ tube may enter through the ostiole of the spermogonium. She does not understand why, if this takes place, it is not seen more frequently, but suggests that

the spermatia may grow at night, in which case she would not have seen them since her material was fixed by daylight. She describes spermatia, each with what she calls short germ tubes extending from both ends, and in some cases germ tubes from two cells appear to have fused.

Structures having a similar appearance are found in the material from which the present study was made. In some cases several spermatia may be seen in a chain connected by an orange-staining unwalled strand. These in the present case are interpreted as being nothing but strands of nectar, which often stain very tenaciously and may have the appearance of mycelial threads. (Fig. 6, G.) The spermatia themselves are frequently attenuated, and are often seen with tails of nectar which give the semblance of germ tubes. The extremely thin spore walls in such cases are sometimes hard to distinguish from the nectar. But what might be a true germination of a spermatium is shown in Figure 4, B. Here is seen a gametophytic hypha grown up between the guard cells, with a deeply stained strand constricted between the guard cells. It is questionable whether the attenuated tip between the guard cells is a continuation of the hypha below or represents a spermatium which has elongated and is fusing with the hyphal tip. A number of similar cases have been seen. Hanna (*25*) reports the formation of germ tubes by spermatia of *Puccinia graminis*. These in one instance attained a length of 15μ. His opinion would seem to be that the spermatia germinate, penetrate the host tissue, and form uninucleate hyphae which grow into the aecial primordia and there fuse with fertile cells, thus initiating the binucleate condition. He does not state whether he has seen any evidence of such a procedure other than the slight germination of spermatia. The writer has obtained no evidence which would suggest that such a method of fertilization occurs in the bean or cowpea rust. A slight capacity for germination or elongation might be very useful in penetrating epidermal cells and making contact with gametophytic hyphae beneath the epidermis.

Another possibility suggested by Allen (*2*) is the fusion of spermatia with paraphyses extending through the ostiole of the spermogonium. Apparently it did not occur to her that these paraphyses, or some of them, might represent trichogynes. Her idea is that a "pycniospore" nucleus may pass down the paraphysis and join a second nucleus at the base of the "pycnium" to form a binucleate hypha which grows into the aecium. As a second possibility it is suggested that two associated spermatia may enter the paraphysis and form a binucleate hypha. She finds no continuous binucleate hyphae running between the spermogonium and the aecium, but does find scattered binucleate cells, which is exactly what should be found if these hyphae were functioning trichogynes. (Fig. 6, G, I.) As a matter of fact erumpent hyphae of the type described above are most frequently found closely associated with the spermogonia, often emerging at points very close to the ostiole. There is no obvious reason why they should not find an exit through the same break in the epidermis formed by the open spermogonium. An attempt was made to find this taking place in the forms studied here, but without success. There is considerable variation in the form of paraphyses protruding from the open spermogonia. Some are variously ruptured and vary in their nuclear content. Spermatia of course are always closely associated with

them, and one might easily imagine that a fusion is taking place in particular cases. But while it is possible that copulation of spermatia with trichogynes may take place at this point very frequently, it can not be said with certainty that it does. Such a concealed method of entrance might account very well for the fact that evidence of copulatory organs has been reported for so few species of rust in addition to the two under present consideration. It is probable, however, that hyphae penetrating the epidermis will be found in other rust fungi upon a study of material at the proper stage. Furthermore, it is not at all impossible that copulation may take place entirely within the host tissue. Such a phenomenon has been reported for *Collema pulposum* (Bernh.) Ach. by Bachmann (*3*). This would be especially feasible where the mycelium from sori of opposite sex has intermingled. Fusions under these conditions would be almost impossible to identify.

Some authors have attached a great deal of importance to the sterile cells in the aecium. To Christman (*10*) they were merely buffer cells which made room for a rapid growth of the fertile cells after fertilization. Blackman (*7, p. 353*) has interpreted them as vestiges of trichogynes. He says, speaking of *Phragmidium violaceum* Wint.:

> Its [the sterile cell's] position above the fertile cell would suggest that it formerly acted as a receptive cell pushing up between the epidermal cells as a trichogyne to which the sticky spermatia could be brought, for example, by insects. Some support is lent to such a view by the fact that occasionally cases are to be found in which the sterile cells do push up between the epidermal cells and swell out above, being merely covered by the cuticle. If development were pushed one stage further and the cuticle pierced, a very effective receptive organ would be the result. [See fig. 7, L.]

Effective receptive organs are present in the two forms of Uromyces studied here, and they often protrude through the epidermis immediately above the aecium. (Fig. 5, F.) But these erumpent hyphae do not correspond to the sterile cells, but are, sometimes in addition to sterile cells, attached to the same fertile cell. These fertile cells with their hyphal branch reaching up to the host epidermis would correspond to the carpogones in the algae. They lie at all angles in the unfertilized aecium, as shown in Figure 2, B. Those nearest the epidermis become functionless as development proceeds below. It appears in this material that the sterile cells are not necessarily sterile but are fertile cells which may possess branches of their own and function as egg cells. As many as three of these are found in a chain. (Fig. 3, G.) The terminal cells are usually vacuolate and in part compose the pseudoparenchyma. Whether more than one fertile cell in such a chain ever produces a chain of aeciospores is uncertain, but they all appear to be essentially of the same structure. Figure 3, E, shows a linkage between basal cells, both of which are maturing spores. This is exactly the appearance that *a* and *b* of Figure 3, G, would probably have after each had begun to produce a chain of spores. It seems reasonable that the binucleate intercalary cell, which is present at the base of the mature aeciospore, corresponds to the secondary branch or trichogyne present on the uninucleate fertile cell. It is often observed that these intercalary cells are not interposed directly between aeciospores but are frequently at a sharp angle. (Fig. 3, D.) This may of course be entirely due to the pressure of surrounding cells.

A great deal of work has been done on the morphology of the aecium (*23*), but very little has been said of the structure of cells in the primordium and at the base of the aecium. Mention has been made of a

tangled mass of mycelium, but no one has seriously attempted to untangle the threads and to show their relationship to the enlarged fertile cells. Although she makes little comment, Allen (*2*) illustrates a condition which is found to be general in the primordia of bean and cowpea rust fungi. She shows hyphae with certain internodes greatly enlarged and supposes that these enlarged cells become the fertile cells. Since she saw no case of nuclei migrating into the fertile cells, she draws no conclusions as to the origin of the binucleate condition. Her drawings show 2-legged cells in the fertilized aecium, yet the fact that she saw no case of cell fusion would indicate that they are not fusion cells.

No observer previous to Allen has more than suggested the peculiar structure and arrangement of hyphae in the primordium. But in regard to the mature or fertilized aecium, they have been more exacting; although here, too, most investigators have failed to show the exact relationship between the basal cells and the hyphae underlying the hymenium. Figure 7, A, C, redrawn from Kursanov (*29*), and Figure 3, A, B, show essentially how the cells (*a*) of Figure 2, F and G, would appear after fertilization. It is obvious that the 2-legged basal cells shown in Figure 1, C, are of a similar origin—that they are not fusion cells but are a development from 2-legged cells present before fertilization.

In 1896, Richards (*35*) described what he termed "fertile hyphae" and "central bodies" found in the aecia of several rusts. (Fig. 10, A, B, C, redrawn from Richards.) No one since has been able to confirm his observations, although several have reported the presence of multinucleate cells and multinucleate hyphae in the aecia of various rust forms. Explanations which are reasonable enough have been offered for multinucleate structures. For example, it has been suggested that they represent the result of nuclear division having proceeded more rapidly than cell division. Figure 11 shows what Richards would probably have interpreted as "central bodies" and "fertile hyphae." Reference to his illustrations will show the similarity. This figure depicts an aecium with two centers or axes of development. In the section the two regions are distinguished from the rest of the aecium by a different intensity of stain and by their remarkable nuclear content. The cells at the upper end of one body show the beginning of a considerable proliferation, while in the other the walls separating it from adjoining cells have dissolved at two points. Walls between surrounding cells appear also to have broken down in some cases. Such a condition is not infrequent in the two forms studied, but is seldom as pronounced as in the case here represented. It probably occurs where conditions are unfavorable for fertilization. Stahl (*37*), Baur (*6*), and Darbishire (*16*) report that carpogones of Collema may disintegrate or return to a vegetative condition where spermatia are not found associated with the trichogynes. Bachmann (*4*) accounts for the absence of such disintegration in her form of *Collema pulposum* on the supposition that greater efficiency of the sexual mechanism results in every carpogone being fertilized. Colley (*12*) emphasizes the constant and normal occurrence of multinucleate cells at the base of the aecium in *Cronartium ribicola* Fischer. He suggests that in rusts of this type the aeciospore chains may arise more often from these placentalike cells than from basal cells resulting from a fusion of only two fertile cells. He con-

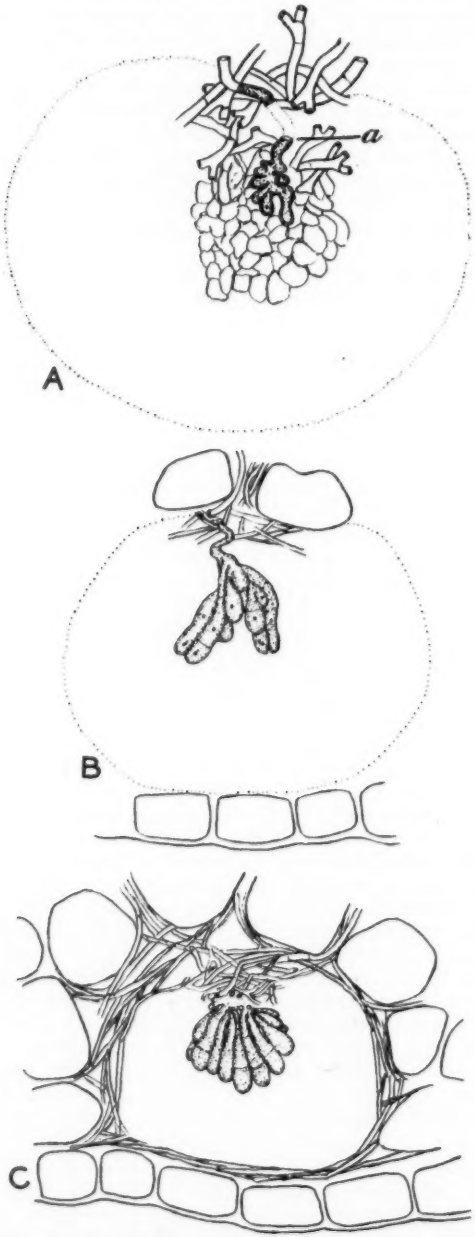

FIGURE 10.—Fertile hyphae and central bodies. × 320. (After Richards, 35)

For explanatory legend see opposite page

cludes that either the extra nuclei in these basal cells degenerate or such basal cells give rise to more than one chain of aeciospores. Basal cells often give rise by proliferation to several chains of binucleate aeciospores, but the extra nuclei in the basal cell do not represent an overdevelopment of a sexual process; they probably represent vegetative fusions and accompanying nuclear migrations in addition to the true fertilization through the trichogyne or trichogenous hypha. The resulting three or more nuclei may divide simultaneously, or two of them may divide while others appear inactive.

Figure 10, A, B, C, redrawn from Richards, and Figure 11, from material of the bean rust, may be interpreted as showing fertile cells which are in process of development while adjacent cells have failed to secure fertilization. The fertilized cells are developing at the expense of the others. The dissolved walls represent nutritive fusions, and the indefinite number of nuclei represent migrations which have in some cases accompanied cell fusion. The proliferated regions show the beginning of several chains of aeciospores which are supported by the increased food supply. It is easy to suppose that in particular cases only one egg cell in an aecium would become fertilized. In others, several would develop, while under very favorable conditions every egg cell would become fertilized, with the result that no proliferation would be seen and probably no cell fusion found.

There are numerous aberrancies among the Uredineae which may not seem to accord with the theory presented in this paper. As a matter of fact the facility with which many of these hitherto meaningless phenomena may be brought into accord by the present theory is most convincing evidence of its plausibility. Dodge has made a number of observations which are highly pertinent, and he has not entirely overlooked their possible significance. He observes the regular position of spermogonia of *Gallowaya pinicola* Arth. beneath stomata (*20*) and the more numerous development of stomata in the region of spermogonia of the orange rusts of Rubus (*18*). But most suggestive is his account of the formation of uninucleate aeciospores in the absence of spermogonia in one form of *Caeoma nitens* Burrill (*19*). There has been considerable difference of opinion as to the time relationship between the spermogonia and the aecia of some coniferous rusts. The observations of Weir and Hubert (*38*) on three species of Cronartium show conclusively the close association of the two spore forms and their probable functional relationship. In some other coniferous rusts it is well established that there is a 12-month interval between the appearance of spermogonia and the maturation of aecia (*1*). Such a delayed maturation following fertilization is not uncommon among fungi.

Some recent work on reproduction in the Ascomycetes (*31*) and in the lichens has brought into question the whole theory of fertilization by means of a trichogyne. If it should be proved that the so-called trichogyne in these forms does not function in reproduction, it is

EXPLANATORY LEGEND FOR FIGURE 10

[These illustrations have been redrawn and inverted]

A.—From an aecidium on *Ranunculus septentrionalis*. Fertile hypha (*a*), which has begun to form branches which will become aeciospore chains. Compare with Figure 11, *d*. B.—*Uromyces caladii*. Later appearance of proliferated basal cell. Compare with Figure 2, A. C.—From an aecidium on *Houstonia caerulea*. Proliferation here is from at least two centers

obvious that some of the arguments presented in this paper would lose much of their potency. Nevertheless, the writer has described what he believes to be the condition present in two forms of the Uredineae, which it is hoped to confirm and clarify by further research. If such should be the case, the presence of a sexual mechanism in the Uredineae similar to that reported for forms of Ascomycetes, lichens, and red algae should be accepted as additional confirmation of the trichogyne theory and an indication of the relationship among these four groups of organisms.

CONCLUSION

The observations recorded here for two forms of Uromyces have shown that gametophytic hyphae grow up through the host epidermis and make contact with spermatia. The evidence is strong that spermatia pass into the ruptured hyphae. . It has been shown that a

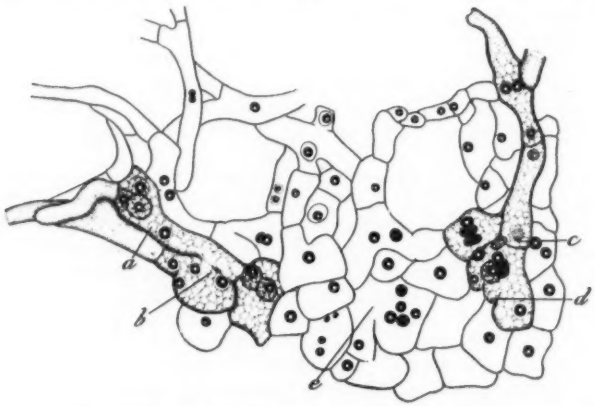

FIGURE 11.—Effect of delayed and incomplete fertilization in *Uromyces appendiculatus*. Section from aecial primordium showing fertilized cells at two centers (*a*, *c*), both representing a series of fertile cells. Vegetative fusions are shown at *b* and *e*, and an early stage in proliferation at *d*. × 830

receptive mechanism is present in the aecial primordium, and that nuclei migrate into the fertile cell through hyphae below and continuous with it. It is suggested that nutritive fusions may take place in a manner similar to those that occur on the carpogone of Dudresnaya (*17*, *34*, *36*). Fusion of adjacent cells in the Uredineae may therefore be compared to fusions with auxiliary cells in forms of red algae. The process of fertilization by fusion of equal gametes, as proposed by Christman (*10*, *11*) and accepted by botanists generally, is brought into question, and the probabilities are that further study will prove the theory no longer tenable. It is obvious that details of the sexual mechanism and phenomena associated with the sexual process will vary with other forms, especially those rusts of the caeomoid type, and those forms, such as the Peridermiums, where the gametophyte is perennial in its host. It may well be that in some species parts of the sexual mechanism have degenerated and a substitute method of fertilization may have developed. In any case, doubtful features of the theory must be dealt with separately.

It is believed that the reasons which led Klebahn (*28*) to conclude that his stomatal hyphae were not functional have since been shown to be unobjectionable.

SUMMARY

A functional sexual mechanism similar to that found in the red algae is believed to be present in the gametophytic stage of the bean-rust organism, *Uromyces appendiculatus*, and the cowpea-rust organism *U. vignae*.

No evidence of the germination or fusion of spermatia has been obtained.

The 2-legged basal cells, similar to those called fusion cells by former observers, are present in the aecial primordium in the uninucleate condition previous to fertilization and are, therefore, not fusion cells.

This 2-legged structure is interpreted as being essentially an egg cell with a foot cell and a trichogyne.

The structures that are believed to function as trichogenous hyphae are much branched and highly septate organs having their terminus at the epidermis of the host leaf where they project through stomata or between epidermal cells and fuse with spermatia transferred by insects, by hand, or by other agencies.

Nuclei were found passing through the cross walls of these hyphae and migrating into the fertile cells in the aecium.

Fusions of parallel hyphae within the aecium do occur, but only under certain conditions, and are probably wholly vegetative or nutritive in function, and may precede, accompany, or follow the true fertilization.

Where all or most of the specially differentiated receptive cells in a single aecium become fertilized there is little or no budding or branching, but in case only one or a few egg cells are fertilized there may result an extensive proliferation which gives the appearance of central bodies and fertile hyphae.

Proliferation is made possible by nutritive fusions with unfertilized cells in the same aecium.

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TWO WILD GRASSES AS HOSTS OF THE HESSIAN FLY, PHYTOPHAGA DESTRUCTOR[1]

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INTRODUCTION

To cope with the Hessian fly most effectively it is essential to know all of its host plants. It is well known that this insect breeds to some extent in barley and rye, and the presence of what was apparently the true *Phytophaga destructor* Say in wild grasses has been reported occasionally. Webster[2] says:

> Many years ago Lindemann, in Russia, found what he determined as the flaxseeds of the Hessian fly on timothy and *Agropyron repens*. Later Mr. Albert Koebele found flaxseeds closely resembling those of the Hessian fly on Elymus, Agrostis, Bromus, and Agropyron in California. Still later Mr. W. J. Phillips found similar flaxseeds in Agropyron about Richmond, Ind., May, 1908. During September of the same year Mr. E. O. G. Kelly found the Hessian fly breeding in abundance in *Agropyron smithii* in the vicinity of Wellington, Kans. * * * On October 25 of the following year Mr. G. I. Reeves found many Hessian fly eggs and larvae in all stages of development up to the flaxseed on *Agropyron repens* and probably also on other varieties of this grass, growing up in the wheat fields in the vicinity of Vancouver, Wash., Mr. C. N. Ainslie found the flaxseed stage of the Hessian fly in *Agropyron tenerum* growing in alleys at Elk Point, S. Dak., June 23, 1913. There is therefore no doubt whatever but that the Hessian fly will breed in Agropyron and perhaps also, in some portions of the country, to a limited extent in Elymus, Agrostis, and Bromus.

Headlee and Parker[3] cite instances of the development of the Hessian fly in *Agropyron smithii*, *A. repens*, and *Elymus canadensis*. Hayhurst[4] found puparia resembling those of the Hessian fly in *A. repens* and reared adults from them. Progeny of these adults were bred from wheat and adults of both generations submitted to E. P. Felt, who stated that he saw no reason for not regarding all of them as the true Hessian fly. Osborn[5] discusses host plants rather extensively and is of the opinion that when forms resembling those of the Hessian fly are found in plants other than wheat their identity should be proven by biologic criteria. That is, in all of these reports there is an element of uncertainty as to whether the flies breeding in the grasses were the true Hessian fly. Osborn's opinion seems well taken. Biological observations are necessary to corroborate the taxonomic evidence that grass-infesting cecidomyiids are identical with *Phytophaga destructor*. If they could be reared from the grass, bred serially into wheat, back into the grass, and out again; or if *P. destructor* from wheat could be carried through a generation in the wild grass and a succeeding generation in wheat, proof of their identity would

[1] Received for publication Feb. 2, 1931; issued May, 1931.

[2] WEBSTER, F. M. THE HESSIAN FLY. U. S. Dept. Agr. Farmers' Bul. 640, 20 p., illus. 1915.

[3] HEADLEE, T. J., and PARKER, J. B. THE HESSIAN FLY. Kans. Agr. Expt. Sta. Bul. 188, p. 83–138, illus. 1913.

[4] HAYHURST, P. QUACK GRASS (AGROPYRON), A HOST OF THE HESSIAN FLY. Jour. Econ. Ent. 2: 231–234. 1909.

[5] OSBORN, H. THE HESSIAN FLY IN THE UNITED STATES. U. S. Dept. Agr., Div. Ent. Bul. 16 (n. s.), 58 p., illus. 1898.

be complete. These processes have been completed experimentally in the greenhouse with *A. repens* and *E. canadensis*, and the object of this paper is to report the results.

BREEDING EXPERIMENTS

In 1925 and 1926 W. B. Cartwright and the writer found larvae and puparia resembling those of the Hessian fly in *Agropyron repens* (L.) Beauv. and *Elymus canadensis* L.[6] near La Fayette, Ind. Attempts were made by the writer to rear succeeding generations in accordance with the procedure outlined above. Flies from these grasses were reared to the puparium stage on wheat, and flies from wheat were reared to the puparium stage on the grass. These rearings were made in outdoor cages, and the entrance of parasites prevented completion of the work.

During 1927 and 1928 the grass-infesting forms practically disappeared from the vicinity of La Fayette. Occasionally a small number of larvae or puparia were found in the wild grasses. Very few adults emerged from such material, and since both sexes were never obtained at the same time it was impossible to do any breeding. It is significant to note that in this same locality there was a coincident comparative scarcity of the Hessian fly in wheat.

Early in 1927 the writer undertook to rear progeny of Hessian flies from wheat and then breed them into *Agropyron repens* and *Elymus canadensis* in the greenhouse.[7] All plants were grown in 4-inch flowerpots to facilitate handling. Adults of *Phytophaga destructor* were reared from puparia taken from volunteer wheat and confined on the grass plants by means of cylindrical cages made of 18-mesh wire screen. They oviposited readily, after which they were removed from the cages and preserved. The young larvae seemed to experience some difficulty in becoming established on the grass plants. Consequently there was a higher mortality of this stage on the grasses than is common on wheat under similar conditions. However, the grass plants soon took on an appearance similar to that characteristic of fly-infested wheat. In 35 days larvae had matured and formed their puparia. The puparia were removed from the plants and placed on moist sand in small tin emergence boxes. Fifteen days later second-generation adults began to emerge and were caged on young wheat plants. After oviposition they were removed from the cages and preserved. The larvae hatching from these eggs established themselves readily on the wheat plants, matured, and formed puparia in an average of 27 days. The puparia were removed from the plants as soon as fully formed and placed on moist sand in tin emergence boxes. In the wheat–*A. repens*–wheat series third-generation adults, reared on wheat, emerged in about 15 days, and in the wheat–*E. canadensis*–wheat series adults of this generation emerged in about 10 days, thus completing the succession of generations on wheat, grass, and wheat. All adults of the third generation were preserved.

The adults of each generation preserved during the course of the experiment and labeled as to generation and host were submitted to C. T. Greene, of the taxonomic unit, United States Bureau of Entomology, who determined them to be *Phytophaga destructor*.

[6] Grasses determined by L. B. Lockwood, of the botany department of Purdue University.

[7] Greenhouse space was available through the courtesy of the Department of Entomology of Purdue University.

In November, 1929, the writer secured from *Agropyron repens* near La Fayette, Ind., 18 puparia resembling those of *Phytophaga destructor*. A few days later a similar find was made in *Elymus canadensis*, and an intensive search yielded about 2 dozen of the puparia. These were placed in emergence boxes and another series of rearings was begun. The method employed was essentially the same as with preceding greenhouse rearings. The flies from the respective grasses were caged separately on young wheat plants and left there until they had oviposited and died. They were then preserved for determination. Larvae hatching from these eggs established themselves readily on the wheat plants and produced a typical infestation in them. Second-generation adults emerging from wheat were in turn caged on young grass plants of the respective varieties from which their grandparents were taken, where they deposited eggs readily. After they had died they were preserved for determination. Larvae hatching from these eggs seemed to experience some little difficulty in becoming established on the grass plants, as was true in the wheat–grass–wheat rearings. However, enough of them did become established to produce a considerable infestation in the grasses, and were reared to the adult stage, thus completing the succession of generations on grass, wheat, and grass. All adults of this generation were preserved for determination.

All specimens preserved during the course of these rearings, labeled as to generation and host, were submitted to C. T. Greene, of the taxonomic unit, United States Bureau of Entomology, who identified them as *Phytophaga destructor*.

During the course of the experiments the question arose as to whether adult females known to be the Hessian fly would oviposit on the wild grasses if young wheat plants were available at the same time. To clear up this point, five pots were seeded with both wheat and *Agropyron repens* and five with both wheat and *Elymus canadensis*. When the plants were all in the 2-leaf stage adult flies reared from wheat were caged on them for oviposition. The flies were placed in the cages about 9 a. m. and left there until the following morning. They were then removed and all eggs counted. The results of the egg count are shown in Tables 1 and 2.

TABLE 1.—*Eggs of Phytophaga destructor deposited on wheat and Agropyron repens in the same cages*

Cage No.	Number of plants exposed		Number of plants infested		Number of eggs deposited on—		Average number of eggs per plant on—	
	Wheat	*A. repens*	Wheat	*A. repens*	Wheat	*A. repens*	Wheat	*A. repens*
1	4	5	4	5	42	94	10.5	18.8
2	6	7	6	7	233	161	38.8	23.0
3	6	5	6	5	53	94	8.8	18.8
4	5	6	5	6	260	278	52.0	46.3
5	5	5	4	5	212	124	42.4	24.8
Total	26	28	25	28	800	751		
Average per plant					30.8	26.8		

TABLE 2.—*Eggs of Phytophaga destructor deposited on wheat and Elymus canadensis in the same cages*

Cage No.	Number of plants exposed		Number of plants infested		Number of eggs deposited on—		Average number of eggs per plant on—	
	Wheat	*E. canadensis*	Wheat	*E. canadensis*	Wheat	*E. canadensis*	Wheat	*E. canadensis*
1	5	5	5	5	91	19	18.2	3.8
2	5	5	5	5	84	54	16.8	10.8
3	5	5	5	5	125	52	25.0	10.4
4	7	7	7	7	67	82	9.5	11.7
5	5	5	5	5	106	23	21.2	4.6
Total	27	27	27	27	473	230		
Average per plant					17.5	8.5		

Even with wheat plants available within the same small cages, no pronounced tendency of the flies to oviposit on the wheat in preference to the grasses was evident. However, it is possible that no great importance should be attached to this observation inasmuch as all oviposition by the Hessian fly in cages takes place under unnatural conditions.

SUMMARY AND CONCLUSIONS

That the Hessian fly (*Phytophaga destructor* Say) can subsist on wild grasses in addition to the cultivated small grains has been alleged for many years. Specimens which have been identified as that species have from time to time been reared from various wild or cultivated grasses, but it had not hitherto been proven that the forms thus derived could be bred back into wheat and afterwards returned to the original host plant. The results of the experiments reported in this paper show that this did occur. The conclusions drawn from this work are as follows:

The true Hessian fly (*Phytophaga destructor*) is able to complete its development on *Agropyron repens* (L.) Beauv. and *Elymus canadensis* L., although not so readily as in wheat.

This insect does sometimes breed in these grasses in nature.

The fly may, under favorable conditions, be able to subsist on these grasses in the absence of wheat.

NUTRIENTS USED FOR MAINTENANCE BY GROWING DAIRY CATTLE [1]

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INTRODUCTION

In connection with experimental studies in which immature dairy cattle are used the need frequently arises for information regarding the maintenance requirements of such animals. A search of the literature reveals a paucity of information on this subject and indicates the desirability of further investigation. Many studies relating to the maintenance requirements of cattle have been conducted since Henneberg and Stohmann (*10, v. 1, p. 17–188*) [2] reported their work in 1858. Important contributions in this field have been made by Wolff (*16*), Kühn and his coworkers (*14*), and Armsby (*1*).

A critical examination of the literature indicates, however, that as yet no comprehensive study has been made in which immature cattle were used as experimental subjects. Almost invariably mature animals have been used and most of them have been of the beef type. As a result, only one of the modern feeding standards, that of Armsby, even attempts to set forth the maintenance requirements of young dairy cattle. This standard was derived by calculation from results secured from mature beef animals, and its application to growing dairy cattle appears questionable. It would be expected that beef animals, because of differences in temperament and conditions of flesh, would offer a different problem from that of animals of the more active dairy type.

The influence of temperament on nutrient requirement was demonstrated by Armsby and Fries (*2*) in trials in which determinations were made over a 3-year period of the maintenance cost of a purebred beef animal and a scrub steer. The latter carried more or less Jersey blood and was of a decidedly more nervous disposition than the other steer. The results showed that the purebred steer required 5.623 therms of net energy for daily maintenance per 1,000 pounds live weight as compared to 6.141 therms required by the scrub steer under similar conditions. It has also been found that fattened steers have a greater maintenance cost per unit of surface area than do unfattened steers (*3*), suggesting a possible difference in this respect between animals of the beef and the dairy types.

The Armsby standard for maintenance of immature dairy cattle is founded on the principle that the net energy requirements of animals vary with the surface area rather than with the weight. Assuming that the net energy requirement at 1,000 pounds live weight is 6.0 therms daily, the requirements at the lower weights are calculated according to the following formula, $X=6.0\left(\frac{W}{1{,}000}\right)^{\frac{2}{3}}$, in which X

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[2] Reference is made by number (italic) to Literature Cited, p. 600.

is the maintenance requirement to be determined at the desired weight W, and 6.0 is the number of therms of net energy required for maintenance at a weight of 1,000 pounds. While the principle involved in this method is subscribed to by such workers as Kellner (*13*), Armsby and Moulton (*4*), and others, some investigators equally well known believe that the fundamental metabolism in animals is in nowise proportional to the body surface but is more nearly determined by live weight.

The question as to the applicability of the Armsby standard to dairy heifers in a practical way has frequently arisen. It was first brought to the attention of the writers as a result of a trial in which a heifer made a gain of approximately a pound a day for six months while receiving a ration the net energy value of which, when calculated by the Armsby method, was approximately the amount set forth as the maintenance requirement in Armsby's tables.

Furthermore, in a recent preliminary study of this problem by the writers (*6*) it was shown that the net energy necessary to maintain uniform weight in dairy-bred calves and heifers in normal flesh is about 90 per cent of that set forth in Armsby's tables. The number of trials represented in this study was too small, however, to afford conclusive results, and for this reason much additional data have been accumulated and are presented here with a more complete study of the problem.

EXPERIMENTAL PROCEDURE

The live-weight method of determining the maintenance requirement was adopted for this study. The many shortcomings of this method are fully appreciated. It is realized that maintenance in its strictest sense can not be determined in this manner. True maintenance, in which there is a perfect balance of matter, can be found only by complete balance trials in which either a respiration chamber or a respiration calorimeter are included as a part of the equipment. These methods, however, are complicated, entail much tedious work, and are very expensive. Even when it is possible to employ these more exact methods the number of animals that can be used is necessarily limited. The live-weight method makes up in part for the inaccuracies by the larger number of animals that can be used.

The chief errors encountered when the live-weight method is employed result from the tendency of the animal body to change in composition without a corresponding change in weight. It is recognized that less food nutrients may be required to maintain a growing animal at constant weight than to maintain actual equilibrium in the animal. The impulse to grow results in some growth taking place when live weight is maintained at a constant figure. Presumably fat oxidized from the tissues may be replaced by water. It is probable that when the trial period is of short duration—40 to 50 days—this possible error will not be sufficient to alter seriously the results obtained. The difficulty of securing accurate live weights is also well known and is a factor to be considered. The live-weight method has, however, in the past been considered sufficiently accurate to give usable results with mature animals in trials extending over long periods of time.

The procedure followed consists in estimating on the basis of a preliminary period, usually a week or more in length, the feed intake

needed for maintenance and then continuing this ration without change throughout the entire experimental period. If the ration as estimated proves to be more liberal than is required for maintenance, a gain will presumably be made until equilibrium is established. If it is too small, the same adjustment will occur at a point somewhere below the original weight.

The present study includes data from 19 animals of various ages used in 31 experimental periods. These periods ranged in length from 15 to 45 days, the average length being 32 days. With immature animals longer periods are believed to be impractical. The animals ranged in weight from 87 to 861 pounds. All were grade or purebred Holsteins. In all cases where an animal was used more than once, a sufficient interval was allowed between the trials to make certain that the subject was in a normal physical condition before it was placed on experiment the second time.

RATIONS

Good quality alfalfa hay and wheat straw served as the sole sources of roughage. The grain ration used consisted of a mixture of ground corn, 4 parts; linseed meal, 1 part; wheat bran, 1 part. Skim milk was fed to the animals at the lower weights.

All feeds used except the skim milk were analyzed by the Division of Agricultural Biochemistry, and from these analyses the nutrient values were calculated by applying the digestion coefficients as given by Henry and Morrison (*12*). No effort was made to control the protein content of the ration except to provide what was considered an adequate supply at all times.

The animals were kept in individual stalls bedded with shavings and allowed the freedom of a dry lot during a part of each day when the weather was not too severe. Water was offered once daily in the barn and was always available in the barn lot. Salt and bone meal were kept in each stall. The weight of each animal was taken each morning before feeding.

RESULTS

Table 1 is a summary of all experimental data. It shows the animals used, their weights, the rations fed during each experimental period, the length of each trial, and the nutrient value of the ration. The nutrient value is presented both in terms of net energy and total digestible nutrients.

As will be observed, two separate net energy values are given for each ration. The first of these represents the net energy content of the ration calculated by the method used by Armsby. The second value differs from the first in that a revised net energy value is applied to the alfalfa hay. Similar revised values for the other feedstuffs in the ration were not available. The revised net energy value applied to the alfalfa hay is the one determined by Forbes and Kriss (*9*) as a result of applying new and improved methods of calculation to the data already collected by Armsby. In this connection it is necessary to point out that Forbes and his coworkers (*7*) in recent studies have found evidence which indicates that the energy value of a ration depends to a great extent on the plane of nutrition at which it is fed. They state that in so far as the revised net energy values are concerned they are based on both submaintenance and supermaintenance feed-

ing, and in the light of present findings as mixed values they approximate "more closely values for body increase than values for maintenance." To date, however, these workers have not presented any definite net energy values based on this conception for feedstuffs when used for maintenance. The revised value suggested for alfalfa hay is therefore presented here tentatively until such time as a more accurate value becomes available. As would be expected, the revised net energy value of the alfalfa hay differs only slightly from that determined by Armsby, being a little higher.

TABLE 1.—*Summary of data regarding weights and rations of 19 dairy calves used in maintenance trials*

Animal No.	Weight of animal at—		Length of trial	Rations fed daily							Total digestible nutrients fed daily	Net energy in ration per day by—	
				Alfalfa hay		Wheat straw		Grain mixture		Skim milk		Armsby method	Revised method
	Start of period	End of period		Weight	Lot No.[d]	Weight	Lot No.[d]	Weight	Lot No.[d]				
	Lbs.	*Lbs.*	*Days*	*Lbs.*		*Lbs.*		*Lbs.*		*Lbs.*	*Lbs.*	*Therms*	*Therms*
E-79	87	87	20							[a] 4.0	0.65	1.16	1.16
E-129	110	114	35	1.00	23					6.0	1.06	1.20	1.25
E-87	114	114	30	.50	6					[a] 4.0	.91	1.33	1.36
E-128	125	128	35	1.00	23					7.0	1.15	1.35	1.40
E-79	135	135	25	1.00	6	0.50	2			7.0	1.36	1.41	1.46
391	233	232	40	1.50	6	1.00	2	0.50	A	5.0	2.02	1.75	1.84
390	249	247	45	2.00	6	1.00	2	.50	A	5.0	2.30	1.95	2.04
391	275	275	35	3.00	6	1.00	2	.50	A	5.0	2.83	2.29	2.44
E-54	284	282	40	2.00	3	1.00	1	1.50	5	6.0	3.13	2.84	2.95
390	308	309	15	3.00	6	1.50	2	1.00	A	3.0	3.23	2.48	2.63
E-15	323	325	35	5.33	1	1.25	1	.73	1		3.91	2.55	2.83
E-14	353	354	35	6.70	1	.74	1	.75	1		4.50	3.01	3.36
E-17	359	360	30	7.11	1	.81	1	.66	[b] 1		4.67	2.95	3.37
E-14	374	373	40	6.24	2	2.08	1	.95	2		4.85	3.12	3.36
E-16	394	394	40	7.38	1	1.10	1	.40	1		4.68	2.95	3.35
E-12	399	399	30	6.55	2	1.47	1	1.08	2		4.91	3.28	3.59
E-15	413	413	45	5.55	2	2.47	1	.53	2		4.30	2.57	2.84
E-65	416	415	35	7.00	5	1.00	2	1.00	[b] B		4.67	3.11	3.54
E-55	455	455	40	7.00	4	1.50	2	1.00	B		4.95	3.21	3.66
E-53	462	461	20	6.50	4	1.00	2	1.50	C		4.94	3.43	3.85
E-54	468	470	40	7.00	4	1.50	2	1.00	[b] B		4.95	3.21	3.66
E-16	521	519	30	7.00	2	2.50	1	.50	2		5.06	3.05	3.38
E-65	536	537	25	7.00	6	2.50	2	.50	[b] D		5.08	3.15	3.48
E-46	588	592	25	9.50	5	2.00	2	[c] 1.00	B		6.31	4.01	4.39
E-55	593	595	25	9.25	5	2.10	2	1.00	B		6.21	3.94	4.49
E-53	663	664	25	10.25	5	2.10	2	1.00	B		6.71	4.25	4.88
415	675	678	20	10.50	23			[c] 2.00	16		6.75	4.95	5.50
E-46	798	799	40	10.50	6	2.50	2	1.50	D		7.74	5.18	5.70
411	833	837	30	12.00	23			[c] 2.00	16		7.52	5.46	6.00
412	840	837	25	12.00	23			[c] 2.00	16		7.52	5.46	6.09
E-53	861	863	35	10.00	6	3.00	2	2.00	D		8.06	5.49	5.98

[a] Whole milk.
[b] Feed of different lot number fed during part of period.
[c] Oats.
[d] The term "lot" is used to designate a certain quantity of feed sampled for chemical analysis. The analyses are given in Table 3.

Figures 1 and 2 present the distributions, indicated by dots, of the net energy provided by the rations as determined according to each of the two methods mentioned. For the sake of comparison Armsby's standard is shown by the solid line in both figures. Similarly the distribution of the total digestible nutrient value of the rations fed during each of the experimental periods is shown in Figure 3. The solid line in this figure indicates the maintenance requirements in terms of total digestible nutrients calculated from Haecker's (*11*) figures for the maintenance of mature cows, namely, 7.925 pounds of

total digestible nutrients per 1,000 pounds live weight.[3] The amount required at the various weights was determined according to the

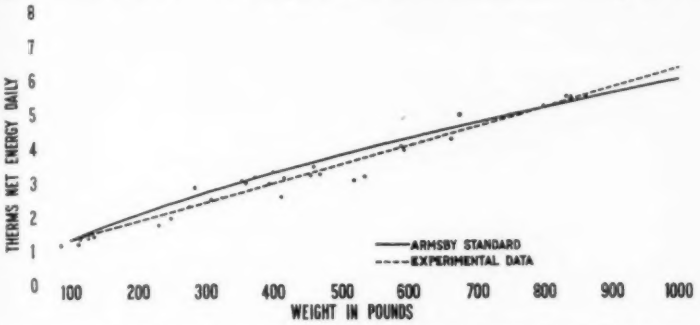

FIGURE 1.—Net energy value of experimental maintenance rations calculated by Armsby's method, compared with the Armsby standard for maintenance

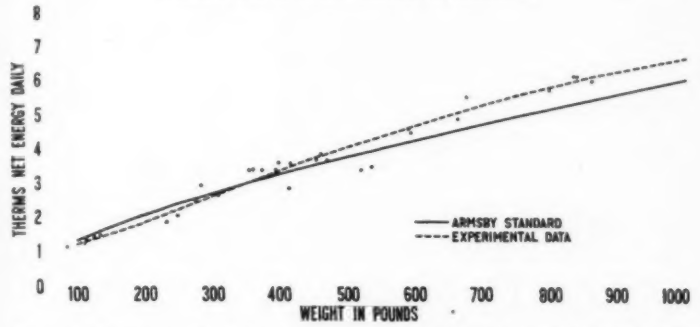

FIGURE 2.—Net energy value of experimental maintenance rations when revised value for alfalfa hay is used, compared with Armsby standard for maintenance

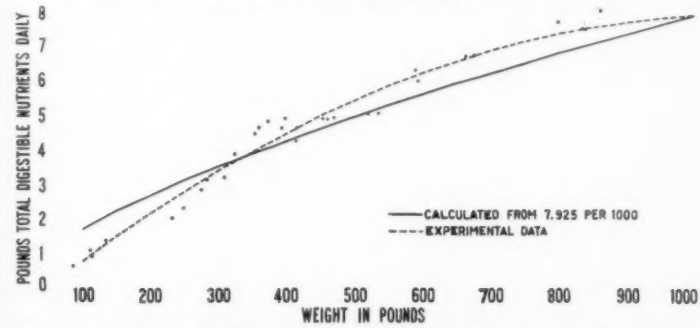

FIGURE 3.—Total digestible nutrients in experimental maintenance rations compared with requirements as calculated from Haecker's figure of 7.925 pounds at 1,000 pounds live weight

formula $X = 7.925\left(\frac{W}{1000}\right)^{2/3}$, in which X is the maintenance requirement to be determined at the desired weight W, and 7.925 is the num-

[3] Morrison in his feeding standard for cows in milk makes use of this figure by Haecker.

ber of pounds of total digestible nutrients required for the maintenance of a cow at a weight of 1,000 pounds live weight. The values indicated by the dotted lines representing the distributions in each figure were determined largely by application of the method of least squares to the data.

TABLE 2.—*Maintenance requirement of dairy calves in terms of therms net energy, and of total digestible nutrients, calculated from the experimental data, as compared with present maintenance standards*

Weight (pounds)	Net energy required on basis of—			Total digestible nutrients required on basis of—	
	Armsby standard	Experimental data		7.925 pounds per 1,000 pounds live weight [a]	Experimental data
		Calculated by Armsby's method	Calculated by revised method		
	Therms	*Therms*	*Therms*	*Pounds*	*Pounds*
100	1.30	1.32	1.20	1.71	0.78
150	1.70	1.59	1.51	2.24	1.50
200	2.05	1.86	1.85	2.71	2.17
250	2.38	2.13	2.21	3.15	2.81
300	2.69	2.40	2.61	3.55	3.41
350	2.98	2.68	2.99	3.94	3.98
400	3.26	2.95	3.35	4.30	4.50
450	3.52	3.22	3.71	4.65	5.00
500	3.78	3.50	4.05	4.99	5.45
550	4.03	3.78	4.37	5.32	5.87
600	4.27	4.05	4.68	5.64	6.24
650	4.50	4.33	4.98	5.95	6.59
700	4.73	4.61	5.26	6.25	6.89
750	4.95	4.89	5.53	6.54	7.16
800	5.17	5.17	5.79	6.83	7.39
850	5.38	5.46	6.03	7.11	7.59
900	5.59	5.74	6.25	7.39	7.74
950	5.80	6.03	6.47	7.66	7.87
1,000	6.00	6.31	6.67	7.93	7.95

[a] This column is calculated by assuming the maintenance requirement to vary in proportion to the two-thirds power of the live weight. Figures varying directly with the live weight may be readily derived.

TABLE 3.—*Chemical analysis of feedstuffs used in maintenance trials*

[Percentage on air-dry basis]

Feedstuff	Lot No. [a]	Moisture	Crude fiber	Crude protein	Ether extract	Nitrogen-free extract	Ash
Alfalfa hay	1	5.52	29.21	16.79	2.83	38.12	7.52
	2	7.08	25.27	16.12	2.84	40.58	8.10
	3	9.63	34.71	13.78	2.30	33.87	5.70
	4	7.11	28.11	20.44	2.31	32.15	9.88
	5	9.30	29.75	16.80	1.68	33.68	8.80
	6	7.06	33.29	15.40	2.11	36.65	5.49
Wheat straw	23	9.38	35.03	17.43	3.14	29.12	5.90
	1	7.30	37.13	4.56	1.69	39.97	9.35
	2	6.34	42.02	4.91	1.56	40.39	4.78
Corn	1	12.75	2.03	9.91	4.41	69.61	1.29
	2	15.34	2.33	9.88	3.76	67.25	1.44
	3	12.47	2.16	10.66	4.71	68.54	1.45
	4	11.46	3.05	12.37	3.71	67.77	1.65
	5	10.50	2.00	10.10	5.00	70.90	1.50
Wheat bran	1	11.65	8.34	14.63	4.66	54.38	6.34
	2	11.71	8.96	13.86	4.86	55.10	5.51
Oats	16	11.27	9.48	12.50	4.20	59.23	3.32
Linseed meal	1	9.71	8.84	34.89	6.25	35.52	4.87
	2	10.08	8.49	36.12	3.09	37.21	5.01
Whole milk		87.20		3.50	3.70	4.90	.70
Skim milk		90.10		3.80	.20	5.20	.70
Grain mixture	1	9.61	4.65	16.59	4.47	60.47	4.21
	2	8.82	6.28	15.81	5.45	59.00	4.63
	5	10.11	5.18	12.41	5.51	63.79	3.00

[a] See footnote *d*, Table 1.

Table 2 expresses the maintenance requirements based on the experimental data in terms of both total digestible nutrients and therms of net energy for immature dairy cattle calculated to regular weight intervals of 50 pounds. Table 3 gives the chemical analysis of all feedstuffs used in the trials and Table 4 shows the manner in which the various grain mixtures were prepared.

TABLE 4.—*Manner in which the grain mixtures were prepared*

Item	Lot number[a] used in making mixture—			
	A	B	C	D
4 parts corn	4	2	5	3
1 part wheat bran	2	1	1	1
1 part linseed meal	2	1	1	2

[a] See footnote *d*, Table 1.

DISCUSSION

Table 1 shows that there is considerable difference in some cases in the requirements for maintenance of animals of approximately equal weights. These variations are perhaps no greater than would be expected in an experiment such as this in which so many factors are involved, many of which are difficult or impossible to control. The effect of difference in condition of animal alone may account for most of the variations observed. The importance of this factor was demonstrated in a previous study by the writers (*6*), in which it was found that animals in high condition may have a maintenance requirement as much as 30 per cent above that of normal animals. It has also been shown by others (*5*, *15*) that after an animal has been on a low level of nutrition for a considerable period the requirements for maintenance are considerably reduced. Although, as has been stated, an attempt was made to have all animals in the same condition of flesh at the time of trial, some differences in this respect probably occurred, for, as is well known, it is very difficult to determine condition accurately in living animals. Other important factors contributing toward the variations indicated are differences in the nutrient value of the feedstuffs not shown by the chemical analyses and differences due to the individuality of the animal.

The recent work of Forbes and Kriss (*9*) previously referred to indicated that the net energy values as expressed by Armsby were on the whole somewhat too low. This finding is confirmed by the comparison of net energy values shown in Table 1. Using the revised net energy value for the alfalfa hay in the ration, the writers found that the requirements for maintenance under the conditions of the experiment were somewhat above those obtained by the Armsby method, especially at the higher weights. New values for the grain and straw would have but slight effect on the requirements of the older animals, as alfalfa hay forms the major portion of their ration.

The greater net energy requirements shown at the higher weights are probably due in part to the greater amount of exercise permitted the older animals. The younger animals while allowed some exercise in the open lot were confined in their box stalls to a greater extent.

It was also noticed that the younger animals showed a greater tendency to lie down and conserve their energy than did the older ones during the period of the trial. On the whole, considering the differences in methods and the character of the animals used, the data here presented show a remarkably close agreement with those obtained by the Armsby standard.

As would be expected when the nutrient values of the experimental rations are expressed in terms of digestible nutrients, similar tendencies are indicated as when net energy is used as the measure. A consideration of Figures 1, 2, and 3 seems to suggest that when the experimental data are expressed in terms of net energy they show a closer agreement with the standards used for comparison than when they are expressed in terms of total digestible nutrients. This is especially apparent at the lower weights. It is possible that the extremely low total digestible nutrient requirement at the lower weights may be due in part at least to a supplementing value of the milk, which formed a large part of the ration at that time. This is a fact not indicated in digestion tables. Moreover, the fact that the younger animals were perhaps more nearly in a state of perfect maintenance during the trials than the older animals might result in a more complete utilization of the nutrients provided. This is suggested by the studies of Forbes, Fries, Braman, and Kriss (*8*) in which the energy metabolism of animals on different planes of nutrition showed that the average rates of utilization of the net energy of the ration for maintenance and body increase were as 1 for maintenance to 0.761 for body increase.

SUMMARY

Experiments were made in which the live-weight method of determining the maintenance requirement of immature dairy animals was used, and the results from a total of 31 trials are reported. The results indicate that when the revised net energy value of alfalfa hay was used the requirements for maintenance under the conditions of the experiment were slightly lower than the requirement of the Armsby standard at weights below 350 pounds and considerably above it at the higher weights. When, however, Armsby's earlier method of calculating the net energy value of feedstuffs was applied to all the feedstuffs in the rations provided the amount of net energy received throughout agreed very closely with the requirements of the Armsby standard. The agreement is less close when the nutrients required and the nutrients received are expressed in terms of total digestible nutrients. In this latter case the comparison is made with Haecker's estimated maintenance requirement of 7.925 pounds of total digestible nutrients per 1,000 pounds live weight.

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NUTRIENT REQUIREMENTS FOR NORMAL GROWTH OF DAIRY CATTLE[1]

By C. H. ECKLES, *Chief*, and T. W. GULLICKSON, *Assistant Dairy Husbandman*, *Division of Dairy Husbandry, Minnesota Agricultural Experiment Station*

INTRODUCTION

The study here reported was undertaken for the purpose of obtaining more accurate information regarding the nutrient requirements of growing dairy cattle. This is a problem that has claimed the attention of several investigators, and considerable information relating to it has been accumulated, especially in recent years; yet the knowledge in this field is still far from complete. It is apparent that not only is such information needed in connection with scientific studies relating to dairy cattle, but it is also of economic importance to the practical dairyman.

PRESENT FEEDING STANDARDS

Feeding standards intended to serve as guides in feeding dairy heifers have been presented by Kellner,[2] Wolff-Lehmann as cited by Henry and Morrison,[3] Armsby,[4] Morrison,[5] and others. A careful analysis of these standards, however, discloses the fact that many of the data from which they were derived are of limited or doubtful value as applied to growing dairy cattle.

Kellner's standard is based on data secured from a few beef steers on rations composed of a limited number of feedstuffs. On the basis of present knowledge it seems apparent that the limited nature of the ration may have been a factor of sufficient importance to affect the accuracy of the results. Further questions may arise because of the small number of animals used and the possibility that beef steers differ from dairy heifers in their growth requirements.

The Armsby standard is based on records secured from a number of sources. Examination reveals that it was calculated by him largely from data taken by other investigators. Using as a basis the net energy value of a unit of gain, he accepts Soxhlet's[6] figure of 1.17 therms as the energy content of 1 pound of increase in live weight of a calf 1 month old. A uniform rate of increase is then assumed up to a maximum of 3.0 therms per pound of gain at 18 months of age. The standard was derived by applying these values to the actual gains secured in cattle at the Missouri station, both beef and dairy types, and adding to it the maintenance requirement. Armsby,[7] in expressing his opinion of it, says "it can not be claimed that the * * * computations are particularly satisfactory. The data

[1] Received for publication Oct. 3, 1930; issued May, 1931. Paper No. 968 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] KELLNER, O. THE SCIENTIFIC FEEDING OF ANIMALS. Authorized transl. by W. Goodwin. 404 p. New York. 1913.

[3] HENRY, W. A., and MORRISON, F. B. FEEDS AND FEEDING; A HANDBOOK FOR THE STUDENT AND STOCKMAN. Ed. 18, 770 p., illus. Madison, Wis. 1923.

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[5] HENRY, W. A., and MORRISON, F. B. Op. cit.

[6] ARMSBY, H. P. Op. cit.

[7] ARMSBY, H. P. Op. cit., p. 402.

are scanty, and the element of personal judgment enters, especially into estimates of the energy value of a unit of increase in live weight."

The Morrison standard[8] is a modification of the Wolff-Lehmann standard, which was prominent a score or more years ago. According to Henry and Morrison,[9] the recommendations in the Morrison standard are based upon data from many sources, including some from European and some from American experiment stations.

It is recognized that the accuracy of any feeding standard can be determined only through its practical application. When present standards have been tested in this manner at this and other stations their inadequacy to serve as guides for feeding growing dairy cattle has been amply demonstrated. Further questions as to their ac-

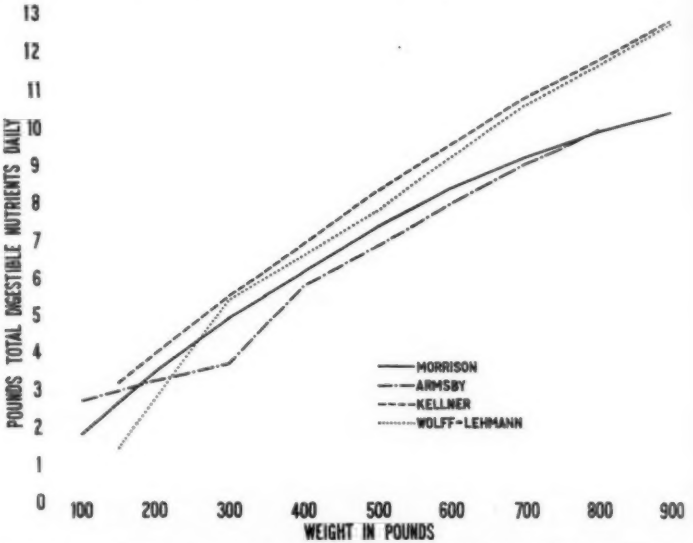

FIGURE 1.—Comparison of various feeding standards for growing dairy heifers; requirements expressed in terms of total digestible nutrients

curacy and practical applicability may arise from the general lack of agreement among them, which is apparent from a study of Table 1 and Figure 1. Since table and graph are self-explanatory a discussion of them will not be necessary. It should be mentioned, however, that the net energy requirements of Armsby and the starch values of Kellner have all been evaluated in terms of total digestible nutrients in order to make the comparison possible.

In the case of the Armsby standard, this was accomplished by selecting typical rations as used under practical conditions and making such small adjustments in the proportion of ingredients as found necessary to meet the requirements of the standard. This procedure was repeated for each 100 increment in weight from 100

[8] In Morrison's standard the recommendations regarding nutrients for an animal of a certain weight are given by two figures between which the requirement of the animal is expected to fall. In this paper the authors have used the average value of the range of these requirements for animals of different weights.

[9] HENRY, W. A., and MORRISON, F. B. Op. cit.

to 800 pounds. The value of these rations was then calculated in terms of total digestible nutrients by the use of the tables given by Henry and Morrison.

Kellner in his standard not only presents the requirements for growth in terms of starch equivalents but also in terms of digestible crude protein, fat, and nitrogen-free extract and crude fiber. The latter were combined by adding to the sum of the digestible crude protein, digestible nitrogen-free extract, and digestible crude fiber, the fat multiplied by 2.25 and expressing the result as "total digestible nutrients."

TABLE 1.—*Requirements for growth of dairy heifers expressed in terms of total digestible nutrients as presented by various feeding standards*

Weight	Total digestible nutrients required by the standard of—			
	Wolff-Lehmann	Kellner [a]	Armsby [b]	Morrison
Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
100	.....	.....	2.70	1.80
150	1.43	3.18	2.98	2.65
200	2.76	.....	3.25	3.50
250	4.09	.....	3.48	4.22
300	5.42	5.51	3.70	4.95
350	6.01	.....	4.75	5.57
400	6.61	.....	5.80	6.20
450	7.21	.....	6.35	6.80
500	7.81	8.33	6.90	7.40
550	8.52	.....	7.45	7.90
600	9.23	.....	8.00	8.40
650	9.93	.....	8.53	8.82
700	10.64	10.85	9.05	9.24
750	11.17	.....	9.50	9.58
800	11.70	.....	9.95	9.92
850	12.23	.....	.....	10.18
900	12.76	12.85	.....	10.44

[a] KELLNER, O. Op. cit., Table III, p. 393.

[b] Total digestible nutrient requirements at different weights determined by calculating typical rations according to Armsby net energy requirement and then evaluating these in terms of total digestible nutrients.

PLAN OF THE EXPERIMENT

In determining the nutrient requirements for growth it is necessary to have a standard with which the growth of the animal may be compared, for obviously the level of feeding will be affected by the rate of growth. Such a standard has been presented by Eckles,[10] and in this study is used as the basis for comparison in all cases.

It is recognized that these normal-growth figures, especially for the Holstein breed, are somewhat low for conditions as maintained in many institution herds and by liberal feeders among purebred cattle breeders. On the other hand, Ragsdale and Regan [11] found that growing dairy animals on Missouri farms were smaller for their age than the figures given in the Eckles normal.

The experiment as conducted consisted of two parts. In the first, which was of a preliminary nature, the object was to determine whether or not the present feeding standards can be relied upon to serve as guides in feeding dairy heifers for normal growth. In this

[10] ECKLES, C. H. THE NORMAL GROWTH OF DAIRY CATTLE. Missouri Agr. Expt. Sta. Research Bul. 36, 23 p., illus. 1920.

[11] RAGSDALE, A. C., and REGAN, M. J. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO DOMESTIC ANIMALS. XIV. (A) MEASUREMENTS OF GROWING HOLSTEIN AND JERSEY CATTLE ON MISSOURI FARMS. (B) PREDICTION CHARTS FOR THE GROWTH OF CATTLE. Missouri Agr. Expt. Sta. Research Bul. 142, 30 p., illus. 1930.

part of the investigation three groups of animals were used. The animals in these groups were provided with nutrients in amounts based on a definite percentage of their requirements according to the Morrison standard. Thus in Group 1 each animal received in its ration approximately 85 per cent of the required amount of nutrients, in Group 2 each received about 100 per cent, and in Group 3 each received approximately 115 per cent. The results were then measured in terms of growth as compared with the normal. Holsteins were used in all three groups, Jerseys only in the second. In the second and main part of the study the object was to determine the nutrients required to secure normal growth. This was accomplished by controlling the amount of nutrient received daily by each animal.

The experiment as conducted was extensive rather than intensive in nature. It was carried on over a period of almost 10 years and data were collected from nearly 50 animals. The animals used were purebreds or high-grade females of the Holstein and Jersey breeds. All animals were in normal health and vigor while on trial.

The ration fed throughout is believed to have been adequate in all essential respects. Young calves were fed whole milk until they were about 2 weeks old, the period of time varying with the vigor and physical condition of the animal. This was followed by skim-milk feeding until the calves were about 6 months old, when hay and grain became the sole source of nutrients. The standard grain mixture used throughout consisted of 4 parts corn meal, 1 part wheat bran, 1 part linseed meal (old process), all by weight. Good quality alfalfa hay was fed to all aninals. Timothy hay and corn silage were used to supplement the legume hay in a few cases. The animals were never allowed to go on pasture.

CARE AND MANAGEMENT OF ANIMALS

The animals were kept in individual pens of a size that made it possible for them to be comfortable at all times. Shavings were used for bedding. Separate mangers were provided for the hay and grain and accurate records kept of the ration fed daily to each individual. Any feed refused was weighed and deducted from the amount fed during the period. Chemical analyses were made of all feeds and their nutrient value determined by using the digestion coefficients as given by Henry and Morrison.[12] Except during extremely cold or stormy weather the animals were allowed to exercise a few hours each day in the barn lot. Weights of all animals were secured every 10 days, and every 30 days weights were taken on three consecutive days, the average of the three being considered the weight for the period. Adjustments and changes in the ration fed were made whenever necessary, usually once every 30 days on the day following the 3-day weighing. As the protein requirement of the growing animal was not a part of this study, care was always taken to provide an adequate supply of it in the ration.

COMPILATION OF DATA

In making the compilations, the data from the preliminary portion of the study were divided into three classes. Class 1 included records from animals receiving 90 per cent or less of their nutrient require-

[12] Henry W. A., and Morrison, F. B. Op. cit., ed. 18, Table II.

ment as prescribed by Morrison, class 2 included the records from animals receiving within 10 per cent; and class 3 those from animals receiving over 110 per cent of the amount of total digestible nutrients required. This plan was followed for each of the two breeds as far as they were represented in each class.

A uniform method of making the calculations was followed throughout. This consisted in calculating the amount of nutrients received by each animal as based on chemical analysis of the ration fed during each period. For animals up to approximately 6 months of age these periods were 10 days in length and each ended on the day the animals were weighed. Beyond this age, periods 30 days in length were used, each ending with the 3-day weigh period. The nutrient requirement during each of these periods, whether 10 days or 30 days in length, was based on the average weight of the animal during the period as determined by taking the average of the weight at the end of the period and that at the end of the previous period.

In all classes in both parts of the study a series of age intervals was established which ranged from 10 days for animals up to approximately 6 months of age to 30 days for the older animals. The average age, the average weight, and the average total digestible nutrients received daily were then used as representative of all the individuals included within each age interval of the series.

In the tables the data are arranged on the basis of regular weight intervals, showing the age of the animal and the total digestible nutrients received daily at the given weights. The total digestible nutrients received over maintenance per pound of gain in weight were derived by subtracting the maintenance requirement from the amount of nutrients received daily at a given weight and dividing the difference by the daily gain in weight. The maintenance requirement indicated for the various weights are those determined by the writers.[13]

RESULTS

CONTROLLED NUTRIENT INTAKE

The results from the trials in which the nutrient intake was controlled are presented in Tables 2–5 and in Figures 2–5. It will be observed that the amount of total digestible nutrients provided was approximately 15 per cent below, 100 per cent of, and 15 per cent above the Morrison standard for the animals in the three groups represented.

The most noteworthy fact concerning these data in view of the difference in the level of nutrition is the small variation in growth between the three groups during the first several months. This appears to indicate that within the limits of the experiment the rate of growth is not affected by the nutrient intake during this period. After this period the rate of growth of each group appears to be in proportion to the nutrient level. It is also apparent that the nutrient intake is sufficient for normal growth in each group up to the age of 6 to 8 months. Beyond this point there is a distinct tendency for the growth of both groups fed at the lower nutrient levels to drop below the normal for several months. The animals in the group on the lowest

[13] GULLICKSON, T. W., and ECKLES, C. H. NUTRIENTS USED FOR MAINTENANCE BY GROWING DAIRY CATTLE. Jour. Agr. Research 42: 593–601, illus. 1931.

level of nutrition showed this tendency most markedly, continuing below normal until more than 13 months old. It appears from these

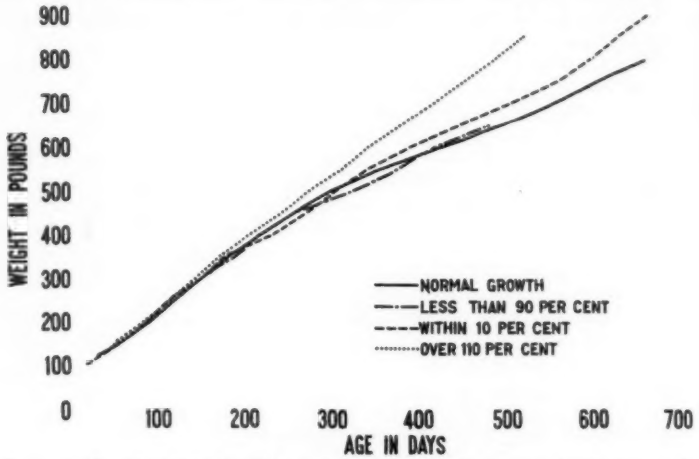

FIGURE 2.—Growth of Holstein heifers, on three planes of feeding, compared with the normal. The animals on the lowest plane of feeding received less than 90 per cent of their requirements as estimated by Morrison, those on the highest plane received 110 per cent or over, while the middle group received approximately 100 per cent

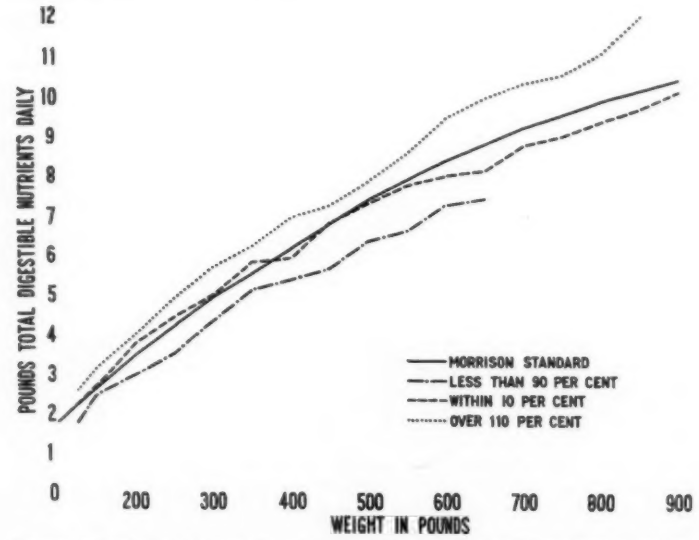

FIGURE 3.—Total digestible nutrients received by Holstein heifers on three planes of feeding. The Morrison standard is included for comparison. The animals on the lowest plane of feeding received less than 90 per cent of their requirements as estimated by Morrison, those on the highest plane received 110 per cent or over, while the middle group received approximately 100 per cent

results that a nutrient level approximately that of the Morrison standard is adequate for normal growth in Holsteins up to about

6 months of age, after which it is slightly too low up to the twelfth month, and beyond this it is more than ample.

While the data for the Jerseys are limited to only one group, the results agree in all essential respects with those of the Holsteins. It

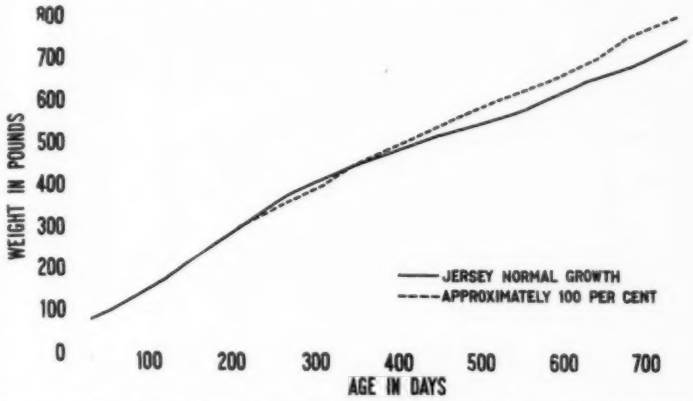

FIGURE 4.—Growth of Jersey heifers fed according to the Morrison standard compared with the normal

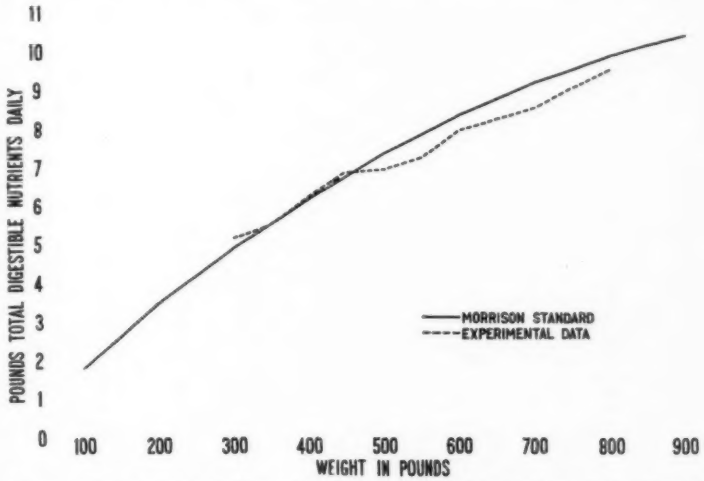

FIGURE 5.—Total digestible nutrients received by Jersey heifers fed approximately 100 per cent of their requirement according to Morrison. The Morrison standard is included for comparison

will be noticed that the nutrient level provided at ages above 1 year is actually nearer to the 90 per cent than to the 100 per cent level, indicating that the provisions of the standard are considerably too high during this period. This statement applies equally well to the Holsteins.

TABLE 2.—*Weights, ages, nutrition planes, and maintenance requirements of Holstein heifers fed 90 per cent or less of their total digestible nutrient requirements as calculated from the Morrison standard*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pounds	*Days*	*Pounds*	*Per cent*		*Pounds*	*Pounds*	*Pounds*
125	33	1.76	79.3	100.0		1.14	
150	54	2.45	92.5	100.0	1.19	1.50	0.80
200	89	3.00	85.7	100.5	1.43	2.17	.58
250	120	3.54	83.9	100.4	1.61	2.84	.45
300	149	4.36	88.1	100.0	1.72	3.41	.55
350	188	5.13	92.1	97.2	1.28	3.98	.90
400	216	5.41	87.3	101.0	1.79	4.50	.51
450	260	5.70	83.8	99.5	1.14	5.00	.61
500	324	6.39	86.4	95.6	.78	5.45	1.21
550	374	6.65	84.2	97.3	1.00	5.87	.78
600	418	7.29	86.8	100.8	1.14	6.24	.92
650	481	7.45	84.5	100.9	.79	6.59	1.09

TABLE 3.—*Weights, ages, nutrition planes, and maintenance requirements of Holstein heifers that received approximately 100 per cent of their total digestible nutrient requirements as calculated from the Morrison standard*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pounds	*Days*	*Pounds*	*Per cent*		*Pounds*	*Pounds*	*Pounds*
100	18.2	1.79	99.4	92.1	1.30	0.78	0.78
150	56.5	2.71	102.3	98.2	1.31	1.50	.92
200	85.5	3.80	108.6	103.4	1.72	2.17	.95
250	113.7	4.43	105.0	104.6	1.77	2.81	.92
300	149.2	5.02	101.4	100.0	1.41	3.41	1.14
350	176.8	5.84	104.8	101.7	1.81	3.98	1.03
400	233.5	5.95	96.0	95.9	.88	4.50	1.65
450	271.2	6.84	100.6	96.3	1.33	5.00	1.38
500	304.7	7.32	98.9	99.0	1.49	5.45	1.26
550	341.6	7.77	98.4	101.8	1.36	5.87	1.40
600	390.7	8.03	95.6	104.5	1.02	6.24	1.75
650	447.7	8.15	92.4	106.4	.88	6.59	1.77
700	507.2	8.78	95.0	106.3	.84	6.89	2.25
750	561.7	9.04	94.4	106.1	.92	7.16	2.04
800	596.7	9.40	94.8	107.8	1.43	7.39	1.41
850	625.4	9.72	95.5	110.5	1.74	7.59	1.22
900	664.9	10.16	97.3	112.4	1.27	7.74	1.91

TABLE 4.—*Weights, ages, nutrition planes, and maintenance requirements of Holstein heifers that were fed 110 per cent or over of their total digestible nutrient requirements as calculated from the Morrison standard*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pounds	*Days*	*Pounds*	*Per cent*		*Pounds*	*Pounds*	*Pounds*
125	38.5	2.58	116.2	95.4		1.14	
150	50.6	3.16	119.2	102.7	2.07	1.50	0.89
200	83.8	4.03	115.1	104.5	1.51	2.17	1.23
250	114.0	4.90	116.1	104.5	1.66	2.81	1.26
300	143.8	5.72	115.6	103.0	1.68	3.41	1.38
350	170.3	6.25	112.2	105.0	1.89	3.98	1.20
400	206.5	6.96	112.3	104.2	1.38	4.50	1.78
450	243.3	7.26	106.8	104.8	1.36	5.00	1.66
500	274.7	7.83	105.2	106.0	1.59	5.45	1.50
550	312.3	8.59	108.7	107.3	1.33	5.87	2.04
600	343.4	9.49	113.0	110.8	1.61	6.24	2.02
650	379.1	9.98	113.2	114.4	1.40	6.59	2.42
700	418.6	10.33	111.8	117.6	1.27	6.89	2.71
750	453.8	10.57	110.3	121.7	1.42	7.16	2.40
800	488.6	11.10	111.9	123.5	1.44	7.39	2.58
850	519.8	12.05	118.4	127.1	1.60	7.59	2.79

TABLE 5.—*Weights, ages, nutrition planes, and maintenance requirements of Jersey heifers that received approximately 100 per cent of their total digestible nutrient requirements as calculated from the Morrison standard*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pound	*Days*	*Pounds*	*Per cent*		*Pounds*	*Pounds*	*Pounds*
300	209	5.18	104.7	99.8		3.41	
350	258	5.51	98.9	96.8	1.02	3.98	1.50
400	310	6.26	101.0	96.3	.96	4.50	1.83
450	350	6.90	101.5	100.4	1.25	5.00	1.52
500	403	6.99	94.5	102.0	.94	5.45	1.64
550	459	7.30	92.4	105.0	.89	5.87	1.61
600	518	8.02	95.5	107.5	.85	6.24	2.09
650	585	8.08	91.6	106.6	.75	6.59	1.99
700	640	8.59	93.0	106.8	.91	6.89	1.87
750	676	9.10	95.0	110.4	1.39	7.16	1.40
800	738	9.57	96.5	109.8	.81	7.39	2.09

CONTROLLED RATE OF GROWTH

The data regarding the animals of both breeds in which growth was controlled at the normal rate are presented in Tables 6 and 7 and Figures 6–9. The most striking feature definitely shown for both breeds is the tendency for the nutrient requirement to be above

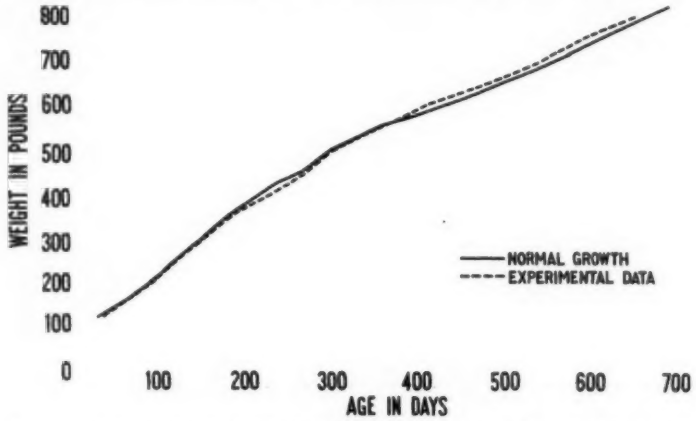

FIGURE 6.—Growth of Holstein heifers fed to grow at the normal rate. The normal growth curve is included for comparison

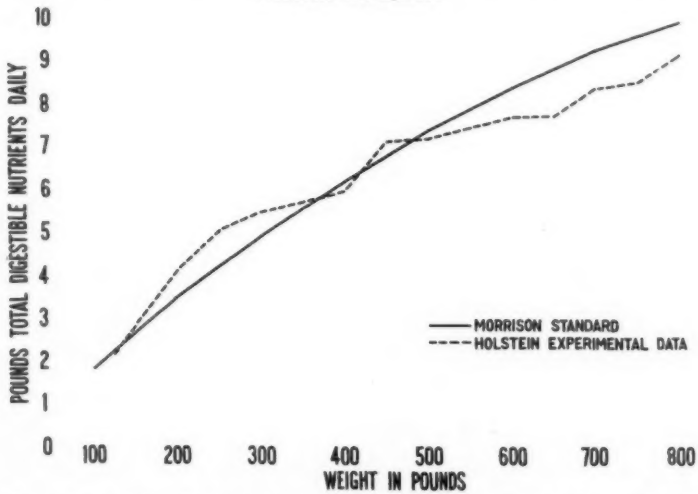

FIGURE 7.—Total digestible nutrients received by Holsteins fed to grow at the normal rate. Morrison standard is included for comparison

that of the Morrison standard for ages below 9 or 10 months. Beyond this point the provisions of the standard are more than ample for normal growth. These results tend further to corroborate what has already been suggested in connection with the preceding group, namely, that the provisions of the Morrison standard are somewhat

too meager for animals under 1 year old and excessive beyond this age.

The results show a tendency on the part of the Jerseys to have a somewhat higher nutrient requirement than the Holsteins at weights

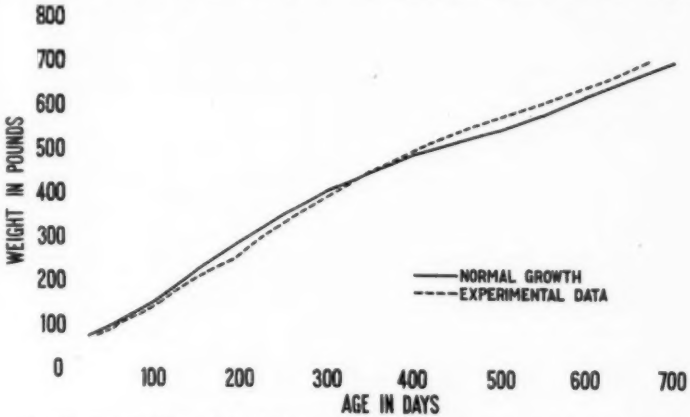

FIGURE 8.—Growth of Jersey heifers fed to grow at the normal rate. The normal growth curve is included for comparison

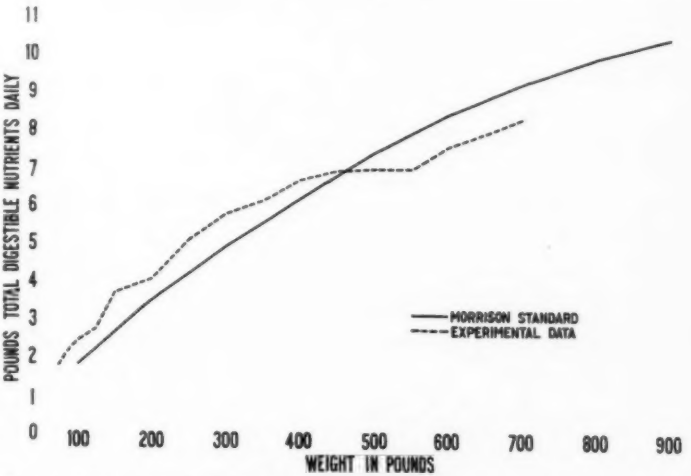

FIGURE 9.—Total digestible nutrients received by Jersey heifers fed to grow at the normal rate. The Morrison standard is included for comparison

up to about 200 pounds. Beyond this point, with few exceptions, the agreement is quite close between the two. This is clearly shown in Figure 10. It will be noted in Tables 6 and 7 that during this time the Jerseys were somewhat smaller with respect to the normal than the Holsteins, making heavier feeding possible as well as desirable.

Table 8 presents the estimated requirements for normal growth based upon the foregoing results from both Holsteins and Jerseys. Morrison's standard is included in the table for comparison. The same data are presented graphically in Figure 10.

NUTRIENT COST OF GAIN IN WEIGHT

It will be noted that the amount of total digestible nutrients required per pound of gain, beyond that needed for maintenance, is

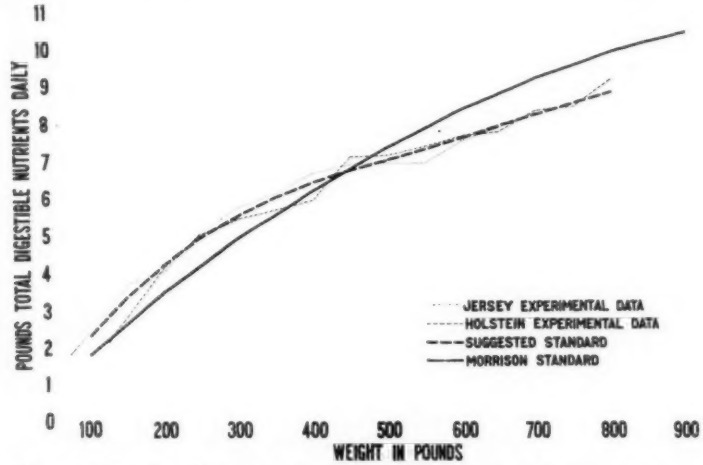

FIGURE 10.—Total digestible nutrients required for normal growth of heifers compared with the mean of the Morrison standard. The total digestible nutrients received by the Holstein and Jersey heifers for normal growth, upon which the suggested standard is based, are also indicated

remarkably uniform for each group in both divisions of the study. There is, as would be expected, some indication, in several of the groups, of an increase in the amount of nutrients required with advance in age. On the other hand when comparisons are made between the various groups of the nutrient cost per pound of gain, considerable difference is observed. Considered in this way economy of gain appears to be correlated with the plane of nutrition provided.

TABLE 6.—*Weights, ages, nutrition planes, and total digestible nutrient requirements of Holstein heifers when their rate of growth was approximately 100 per cent of the normal throughout*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pound	*Days*	*Pounds*	*Per cent*		*Pounds*	*Pounds*	*Pounds*
125	38	2.15	96.8	95.7		1.14	
150	58	2.83	106.8	97.0	1.25	1.50	1.06
200	93	4.13	118.0	97.6	1.43	2.17	1.37
250	122	5.04	119.4	99.0	1.72	2.81	1.30
300	151	5.47	110.5	98.8	1.72	3.41	1.20
350	187	5.70	102.3	97.7	1.39	3.98	1.24
400	231	5.96	96.1	96.6	1.14	4.50	1.28
450	272	7.13	104.9	96.1	1.22	5.00	1.75
500	302	7.19	97.2	99.4	1.67	5.45	1.04
550	358	7.43	94.1	98.9	.89	5.87	1.75
600	408	7.70	91.7	102.2	1.00	6.24	1.46
650	479	7.71	87.4	101.2	.70	6.59	1.60
700	541	8.36	90.5	101.9	.81	6.89	1.81
750	588	8.48	88.5	102.2	1.06	7.16	1.25
800	650	9.18	92.5	101.4	.81	7.39	2.21

TABLE 7.—*Weights, ages, nutrition planes, and total digestible nutrient requirements of Jersey heifers when their rate of growth was approximately 100 per cent of the normal throughout*

Weight	Age	Total digestible nutrients received per day	Plane of nutrition	Percentage of normal weight	Gain in weight per day	Total digestible nutrients required for maintenance	Total digestible nutrients over maintenance required per pound of gain in weight
Pound	*Days*	*Pounds*	*Per cent*		*Pounds*	*Pounds*	*Pounds*
75	36	1.77	128.7	91.7			
90	53	2.24	137.4	91.7	0.88		
100	61	2.41	133.9	94.2	1.25	0.78	1.30
125	83	2.75	123.9	94.8	1.14	1.14	1.41
150	106	3.70	139.7	94.9	1.09	1.50	2.02
200	146	4.08	116.6	92.8	1.25	2.17	1.53
250	194	5.09	120.6	89.4	1.04	2.81	2.19
300	226	5.79	117.0	93.1	1.56	3.41	1.53
350	262	6.13	110.1	95.5	1.39	3.98	1.55
400	306	6.69	107.9	97.1	1.14	4.50	1.92
450	348	6.94	102.1	100.8	1.19	5.00	1.63
500	399	6.97	94.2	102.7	.98	5.45	1.55
550	461	6.97	88.2	104.8	.81	5.87	1.36
600	540	7.58	90.2	104.9	.63	6.24	2.13
650	611	7.92	89.8	103.0	.70	6.59	1.90
700	670	8.30	89.8	103.7	.85	6.89	1.66

TABLE 8.—*Total digestible nutrients found to be required for the normal growth of Jersey and Holstein dairy heifers as compared with the Morrison standard*

Weight	Total digestible nutrients received daily during experiments		Total digestible nutrients required daily by—	
	Jerseys	Holsteins	Experimental trial	Morrison standard[a]
Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
100	2.41	----------	2.32	1.80
150	3.70	2.83	3.36	2.65
200	4.08	4.13	4.23	3.50
250	5.09	5.04	4.96	4.22
300	5.79	5.47	5.55	4.95
350	6.13	5.70	6.03	5.57
400	6.69	5.96	6.44	6.20
450	6.94	7.13	6.77	6.80
500	6.97	7.19	7.06	7.40
550	6.97	7.43	7.34	7.90
600	7.58	7.70	7.65	8.40
650	7.92	7.71	7.96	8.82
700	8.30	8.36	8.26	9.24
750	----------	8.48	8.56	9.58
800	----------	9.18	8.85	9.92

[a] In the Morrison standard a range is given within which the requirement should fall. The figures used in this table are a mean of the two extremes as given by Morrison.

SUMMARY

Comparisons are made between the Wolff-Lehmann, Kellner, Armsby, and Morrison feeding standards for growing dairy heifers. Experimental data are presented showing the nutrient requirements for normal growth of dairy heifers based on the normal-growth weights of Eckles, and also regarding the rate of growth of dairy heifers at different levels of the nutrient requirement as based on the Morrison standard.

The Morrison standard is found to be too low for normal growth of dairy heifers up to about 1 year of age and too high beyond that age.

The total digestible nutrient requirements found to be necessary for the normal growth of dairy heifers are shown.

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